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Lecture Notes

Microbiology/Immunology

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VISIT…

MICROBIOLOGY

Author

Louise Hawley, Ph.D.
Assistant Professor
Department of Medical Microbiology and Immunology
University of Minnesota
Duluth School of Medicine
Duluth, MN

Coauthor

Don J. Dunn, Ph.D.
Professor, Microbiology and Immunology
Oklahoma University Health Sciences Center
Oklahoma City, OK

Contributors

Mary Ruebush, Ph.D.
Microbiology/Immunology
Adjunct Professor of Microbiology
Montana State University
Bozeman, MT

Kenneth H. Ibsen, Ph.D.
Director of Academic Development
Kaplan Medical
Professor Emeritus Biochemistry
University of California-Irvine
Irvine, CA

IMMUNOLOGY

Author

Mary Ruebush, Ph.D.
Microbiology/Immunology
Adjunct Professor of Microbiology
Montana State University
Bozeman, MT

Contributors

Don J. Dunn, Ph.D.
Professor Emeritus
Oklahoma University Health Sciences Center
Oklahoma City, OK

Thomas F. Lint, Ph.D.
Professor of Immunology and Microbiology
Rush Medical College
Chicago, IL

Gerald C. Miller, Ph.D. and M.S.
Chief of Immunology
Regional Medical Laboratory

Kim Moscatello, Ph.D.
Assistant Professor of Microbiology and Immunology
Lake Erie College of Osteopathic Medicine
Erie, PA

Executive Director of Curriculum
Richard Friedland, M.D.

Director of Publishing and Media
Michelle Covello

Medical Illustrator and Cover Art
Christine Schaar

Managing Editor
Kathlyn McGreevy

Production Artist
Michael Wolff

Production Editor
William Ng

Cover Design
Joanna Myllo

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SECTION I

Microbiology

General Microbiology

1

What the USMLE Requires You to Know

- Differences among viruses, fungi, bacteria, and parasites
- Differences between eukaryotic and prokaryotic cells
- Important normal flora
- Major mechanisms of pathogenicity

MAJOR MICROBIAL GROUPS

Table I-1-1. Comparison of Medically Important Microbial Groups

Characteristic	Viruses*	Bacteria	Fungi	Parasites
Diameter**	Minute (0.02–0.3 μ)	Small (0.3–2 μ)	3–10 μ	15–25 μ (trophozoites)
Cell type	**Acellular** (not cell) No nucleus	**Prokaryotic cells** Nucleoid region: no nuclear membrane	**Eukaryotic cells** Nucleus with nuclear membrane	**Eukaryotic cells**
	DNA or RNA 1 nucleocapsid except in segmented or diploid viruses	DNA and RNA 1 chromosome **No histones**	DNA and RNA More than 1 chromosome	
	Replicates in host cells	DNA replicates continuously	G and S phases	
		Exons, no introns	Introns and exons	
	Some have polycistronic mRNA*** and post translational cleavage	Mono- and polycistronic mRNA	Monocistronic RNA	
	Uses host organelles; obligate intracellular parasites	No membrane bound organelles	Mitochondria and other membrane-bound organelles	
	No ribosomes	**70S** ribosomes (30s+50s)	**80S** ribosomes (40S+60S)	
Cellular membrane	Some are enveloped: but no membrane function	Membranes have **no sterols except Mycoplasmas,** which have cholesterol	Membrane **ergosterol** is major sterol.	Sterols such as cholesterol
Cell wall	No cell wall	**Peptidoglycan**	Complex carbohydrate **cell wall: chitin,** glucans, or mannans	No cell wall
Replication	Make and assemble viral components	**Binary fission (asexual)**	Cytokinesis with mitosis / meiosis	Cytokinesis with mitosis / meiosis

*Besides viruses, **two other acellular forms exist:**
- Viroids: obligate intracellular but acellular parasites of plants; naked RNA; no human diseases.
- Prions: acellular particles associated with Kuru, etc.; insensitive to nucleases.
  Abnormal prion proteins (PrP) modify folding of normal prion-like proteins found in the body (coded for by human genes).

If the **diameter of a cell described in a clinical case is >2 μ, then it is **probably a eukaryotic cell.**

***Polycistronic mRNA carries the genetic code for several proteins. (It has multiple Shine-Dalgarno sites.)

Epidemiology

Normal Flora

- Is found on body surfaces contiguous with the outside environment
- Is semi-permanent, varying with major life changes
- Can cause infection
  - if misplaced, e.g., fecal flora to urinary tract or abdominal cavity, or skin flora to catheter
  - or, if person becomes compromised, normal flora may overgrow (oral thrush)
- Contributes to health
  - protective host defense by maintaining conditions such as pH so other organisms may not grow
  - serves nutritional function by synthesizing: K and B vitamins

In A Nutshell

Definitions

Carrier: person colonized by a potential pathogen without overt disease.

Bacteremia: bacteria in bloodstream without overt clinical signs.

Septicemia: bacteria in bloodstream (multiplying) with clinical symptoms.

Table I-1-2. Important Normal Flora

Site	Common or Medically Important Organisms	Less Common but Notable Organisms
Blood, internal organs	None, generally sterile	
Cutaneous surfaces including urethra and outer ear	*Staphylococcus epidermidis*	*Staphylococcus aureus,* Corynebacteria (diphtheroids), streptococci, anaerobes, e.g., peptostreptococci, yeasts (*Candida* spp.)
Nose	*Staphylococcus aureus*	*S. epidermidis,* diphtheroids, assorted streptococci
Oropharynx	**Viridans streptococci** including *Strep. mutans*[1]	Assorted streptococci, **nonpathogenic *Neisseria*, nontypeable**[2] ***Haemophilus influenzae***
Gingival crevices	Anaerobes: *Bacteroides, Prevotella, Fusobacterium, Streptococcus, Actinomyces*	
Stomach	None	
Colon (microaerophilic/ anaerobic)	Babies; breast-fed only: *Bifidobacterium* Adult: ***Bacteroides****/Prevotella* (Predominant organism) *Escherichia* *Bifidobacterium*	*Lactobacillus,* streptococci *Eubacterium, Fusobacterium, Lactobacillus,* assorted Gram-negative anaerobic rods, *Enterococcus faecalis* and other streptococci
Vagina	***Lactobacillus***[3]	Assorted streptococci, Gram-negative rods, diphtheroids, yeasts

[1] ***S. mutans*** secretes a biofilm that glues it and other oral flora to teeth, producing **dental plaque.**

[2] (Nontypeable for *Haemophilus* means no capsule.)

[3] Group B streptococci colonize vagina of 15–20% of women and may infect the infant during labor or delivery, causing septicemia and/or meningitis (as may *E. coli* from fecal flora).

PATHOGENICITY (INFECTIVITY AND TOXICITY) MAJOR MECHANISMS

Colonization

(Important unless organism is traumatically implanted.)

Adherence to cell surfaces involves

- **Pili/fimbriae:** primary mechanism in most gram-negative cells.
- **Teichoic acids:** primary mechanism of gram-positive cells.
- **Adhesins:** colonizing factor adhesins, pertussis toxin, and hemagglutinins.
- **IgA proteases:** cleaved Fc portion may coat bacteria and bind them to cellular Fc receptors.

Partial Adherence to inert materials, **biofilms**: *Staph. epidermidis, Streptococcus mutans*

Avoiding immediate destruction by host defense system:

- **Anti-phagocytic surface components** (inhibit phagocytic uptake):
  - **Capsules**/slime layers:
    ***Streptococcus pyogenes* M protein**
    ***Neisseria gonorrhoeae* pili**
    ***Staphylococcus aureus* A protein**
- **IgA proteases,** destruction of mucosal IgA: *Neisseria, Haemophilus, S. pneumoniae*

"Hunting and gathering" needed nutrients:

- **Siderophores** steal (chelate) and import iron.

Note

Mnemonic

Streptococcus pneumoniae
Klebsiella pneumoniae
Haemophilus influenzae
Pseudomonas aeruginosa
Neisseria meningitidis
Cryptococcus neoformans

(Some Killers Have Pretty Nice Capsules)

Ability to Survive Intracellularly

- **Evading intracellular killing by professional phagocytic cells** allows intracellular growth:
  - *M. tuberculosis* survives by inhibiting phagosome-lysosome fusion.
  - *Listeria* quickly escapes the phagosome into the cytoplasm **before** phagosome-lysosome fusion.
- **Invasins**: surface proteins that allow an organism to bind to and invade normally non-phagocytic human cells, escaping the immune system. Best studied invasin is on *Yersinia pseudotuberculosis* (an organism causing diarrhea).
- Damage from viruses is largely from intracellular replication, which either kills cells, transforms them or, in the case of latent viruses, may do no noticeable damage.

Note

Intracellular organisms

- Elicit different immune responses
- Different pathology
- Different antibiotics
- Different cultural techniques

Inflammation or Immune-Mediated Damage

Examples

- **Cross-reaction of bacteria-induced antibodies with tissue antigens** causes disease. Rheumatic fever is one example.
- **Delayed hypersensitivity and the granulomatous response** stimulated by the presence of intracellular bacteria is responsible for neurological damage in leprosy, cavitation in tuberculosis, and fallopian tube blockage resulting in infertility from *Chlamydia* PID (pelvic inflammatory disease).

- **Immune complexes** damage the kidney in post streptococcal acute glomerulonephritis.
- **Peptidoglycan-teichoic acid** (large fragments) of Gram-positive cells:

  Serves as a structural toxin released when cells die.

  Chemotactic for neutrophils.

Physical Damage

Swelling from infection in a fixed space damages tissues; examples: meningitis and cysticercosis.

Large physical size of organism may cause problems; example: *Ascaris lumbricoides* blocking bile duct.

Aggressive tissue invasion from *Entamoeba histolytica* causes intestinal ulceration and releases intestinal bacteria, compounding problems.

TOXINS

Toxins may aid in invasiveness, damage cells, inhibit cellular processes, or trigger immune response and damage.

Structural Toxins

- **Endotoxin** (Lipopolysaccharide = LPS)
  - LPS is part of the **Gram-negative outer membrane.**
  - **Toxic portion is lipid A:** generally not released (and toxic) until death of cell. Exception: *N. meningitidis,* which over-produces outer membrane fragments.
  - **LPS is heat stable** and not strongly immunogenic so it **cannot be converted to a toxoid.**
  - Mechanism

    LPS activates macrophages, leading to release of TNF-alpha, IL-1, and IL-6.

    IL-1 is a major mediator of fever.

    Macrophage activation and products lead to tissue damage.

    Damage to the endothelium from **bradykinin-induced vasodilation leads to shock.**

    Coagulation (DIC) is mediated through the **activation of Hageman factor.**
- **Peptidoglycan, Teichoic Acids**
- **Exotoxins**
  - are **protein toxins,** generally **quite toxic** and **secreted by** bacterial **cells** (some Gram +, some Gram –)
  - **can be modified** by chemicals or heat to produce a **toxoid** that still is **immunogenic, but no longer toxic** so can be used as a vaccine
  - **A-B** (or "two") **component** protein toxins

    B component **binds** to specific cell receptors to facilitate the internalization of A.

    A component is the **active (toxic) component** (often an enzyme such as an ADP ribosyl transferase).

    Exotoxins may be subclassed as enterotoxins, neurotoxins, or cytotoxins.
  - **Cytolysins:** lyse cells from outside by damaging membrane.

    ***C. perfringens* alpha toxin** is a **lecithinase.**

    ***Staphylococcus aureus* alpha toxin** inserts itself to form **pores** in the membrane.

Table I-1-3. Major Exotoxins

	Organism (Gram)	Toxin	Mode of Action	Role in Disease
Protein Inhibitors	*Corynebacterium diphtheriae* (+)	Diphtheria toxin	ADP ribosyl transferase; inactivates EF-2; 1′ targets: heart/nerves/ epithelium	Inhibits eukaryotic cell protein synthesis
	Pseudomonas aeruginosa (–)	Exotoxin A	ADP ribosyl transferase; inactivates EF-2; 1′ target: liver.	Inhibits eukaryotic cell protein synthesis
	Shigella dysenteriae (–)	Shiga toxin	Interferes with 60S ribosomal subunit	Inhibits protein synthesis in eukaryotic cells. Enterotoxic, cytotoxic, and neurotoxic
	Enterohemorrhagic *E. coli* (EHEC)	Verotoxin (a shiga-like toxin)	Interferes with 60S ribosomal subunit	Inhibits protein synthesis in eukaryotic cells
Neurotoxins	*Clostridium tetani* (+)	Tetanus toxin	Blocks release of the inhibitory transmitters glycine and GABA	Inhibits neurotransmission in inhibitory synapses
	Clostridium botulinum (+)	Botulinum toxin	Blocks release of acetylcholine	Inhibits cholinergic synapses
Endotoxin Enhancers	*Staphylococcus aureus* (+)	TSST-1	Pyrogenic, decreases liver clearance of LPS, superantigen	Fever, increased susceptibility to LPS, rash, shock, capillary leakage
	Streptococcus pyogenes (+)	Exotoxin A, a.k.a.: erythrogenic or pyrogenic toxin	Similar to TSST-1	Fever, increased susceptibility to LPS, rash, shock, capillary leakage, cardiotoxicity
cAMP Inducers	Enterotoxic *Escherichia coli* (–)	Heat labile toxin (LT)	LT stimulates an adenylate cyclase by ADP ribosylation of GTP binding protein	Both LT and ST promote secretion of fluid and electrolytes from intestinal epithelium
	Vibrio cholerae (–)	Cholera toxin	Similar to *E. coli* LT	Profuse, watery diarrhea
	Bacillus anthracis (+)	Anthrax toxin (3 proteins make 2 toxins)	EF = edema factor = adenylate cyclase LF = lethal factor PA = protective antigen (B component for both)	Decreases phagocytosis; causes edema, kills cells
	Bordetella pertussis (–)	Pertussis toxin	ADP ribosylates G_i, the negative regulator of adenylate cyclase → increased cAMP	Histamine-sensitizing Lymphocytosis promotion Islet activation
Cytolysins	*Clostridium perfringens* (+)	Alpha toxin	Lecithinase	Damages cell membranes; myonecrosis
	Staphylococcus aureus (+)	Alpha toxin	Toxin intercalates forming pores	Cell membrane becomes leaky

Chapter Summary

The size, cell type, cellular membrane, cell walls, and modes of replication of viruses, bacteria, fungi, and protozoan parasites are compared, as are the differences between prokaryotes and eukaryotes. There is also a brief consideration of the properties and pathogenicity of prions and plant viroids.

The important normal microflora typically associated with various body sites are described.

For the successful induction of pathogenicity, microorganisms must be infective and toxic. Infectivity requires colonization, which in turns requires adhesion, the avoidance of destruction by the host, and the invasion of cells. Modes of damage include damage caused by immune-mediated inflammation, physical-damage–associated tissue invasion, and/or production of endo- or exotoxins.

Review Questions

1. Your laboratory isolates an entirely new and unknown pathogen from one of your patients, which has all the characteristics of an aerobic filamentous fungus except that the ribosomes are prokaryotic. Unfortunately, your patient with this pathogen is very ill. Which agent would most likely be successful in treating your patient?
   A. Third generation of cephalosporins
   B. Isoniazid
   C. Metronidazole
   D. Careful limited usage of Shiga toxin
   E. Tetracycline

2. Mitochondria are missing in
   A. Filamentous fungi
   B. Protozoan parasites
   C. Viruses
   D. Yeasts
   E. Cestodes

Answers

1. **Answer: E.** The cephalosporin that inhibits prokaryotic cell peptidoglycan cross linkage will not likely be effective against the complex carbohydrate cell wall. Isoniazid, which appears to inhibit mycolic acid synthesis, also would not likely work. Metronidazole would not work on an aerobic organism. Shiga toxin is only effective against eukaryotic ribosomes. Tetracycline (the correct answer) would have the greatest chance of success. However, it may not be taken up by the cell, or the cell could have an effective pump mechanism to get rid of it quickly.

2. **Answer: C.** Mitochondria are found only in eukaryotic organisms so both viruses and bacteria lack them.

Medically Important Bacteria

2

What the USMLE Requires You to Know

The type of disease (major diseases) from presenting symptoms

- You must know the common etiologic agents of the disease and be able to determine the causative agent of the particular case from case clues.
- No distinguishing clues given? Know most common agent(s).
- Epidemiologic clues, symptomatic clues, or organism information given? Know the specific agent.
- Be able to answer basic science questions about disease or organism, predisposing conditions, epidemiology, mechanism of pathogenicity, and major tests used in identification.

The basic science used as clues or tested directly

Morphology

- Gram reaction, basic morphology, motility (*Listeria*), spore formation (*Bacillus* and *Clostridium*)

Physiology

- Obligate aerobes/anaerobes
- A few specific fermentations
- A few specific enzymes (oxidase, urease, catalase, coagulase, superoxide dismutase, hemolysins)
- How bacterial cells grow, divide, and die

Bacterial structures

- Their composition, function, and role in disease

Determinants of pathogenicity

- Toxins
- Factors aiding in invasiveness, pathogenicity, or immune system evasion
- Intracellular parasites (obligate and facultative)

Epidemiology/transmission

- Know how each major disease is acquired
- Particularly important for organisms with animal or arthropod vectors

(Continued)

Note

Nomenclature

Latin bacterial **family** names have "**-aceae,**" e.g., Enterobacteriaceae.

Genus and species names are **italicized** and **abbreviated,** e.g., *Enterobacter aerogenes* = *E. aerogenes*.

What the USMLE Requires You to Know (*continued*)

Laboratory diagnosis

- Serologic/skin tests: specific serology for syphilis
- Stains: acid fast and Gram
- Unusual growth requirements
- Specific media
- Steps in Gram stain and acid fast stain

Diseases

- Common presenting symptoms
- Stages of multistage diseases
- Common complications

Treatment (drug of choice – pharmacology)/prevention

(vaccination, public health, and prophylaxis, where regularly used)

(Basically the same for all other pathogens, too!)

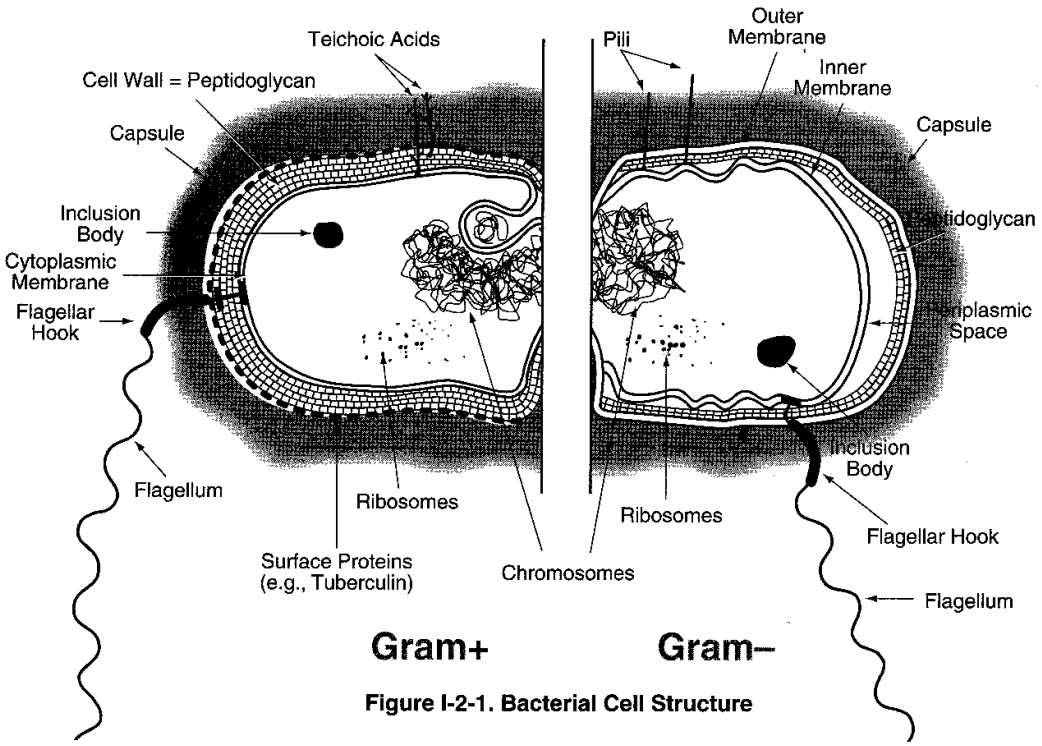


Figure I-2-1. Bacterial Cell Structure

Table I-2-1. Bacterial Envelope (All the Concentric Surface Layers of the Bacterial Cell)

Envelope Structure	Gram + or –	Chemical Composition	Function
Capsule (Non-essential) = Slime = Glycocalyx	Both Gram + Gram –	Polysaccharide gel*	Pathogenicity factor protecting against phagocytosis until opsonized; immunogenic**
Outer membrane	Gram-negative only	Phospholipid/proteins: Lipopolysaccharide Lipid A Polysacccharide	Hydrophobic membrane: LPS = endotoxin Lipid A = toxic moiety PS = immunogenic portion
		Outer membrane proteins	Attachment, virulence, etc.
		Protein porins	Passive transport
Cell wall = peptidoglycan	Gram + (thick) Gram – (thin)	Peptidoglycan-open 3-D net of: N-acetyl-glucosamine N-acetyl-muramic acid amino acids (DAP)	Rigid support, cell shape, and protection from osmotic damage Synthesis inhibited by penicillins and cephalosporins Confers Gram reaction
	Gram-positive only	Teichoic acids***	Immunogenic induces TNF-alpha, IL-1 Attachment
	Acid-fast only	Mycolic acids	Acid-fastness Resistance to drying and chemicals
Periplasmic space	Gram-negative only	"Storage space" between the inner and outer membranes	Enzymes to break down large molecules, (β-lactamases) Aids regulation of osmolarity
Cytoplasmic membrane = inner membrane = cell membrane = plasma membrane	Gram + Gram –	Phospholipid bilayer with many embedded proteins	Hydrophobic cell "sack" Selective permeability and active transport Carrier for enzymes for: Oxidative metabolism Phosphorylation Phospholipid synthesis DNA replication Peptidoglycan cross linkage Penicillin Binding Proteins (PBPs)

* Except *Bacillus anthracis*, which is a polypeptide of poly D-glutamate.
** Except *S. pyogenes* (hyaluronic acid) and type B *N. meningitidis* (sialic acid), which are nonimmunogenic.
*** Teichoic acid: polymers of ribitol or glycerol, bound to cell membrane or peptidoglycan.

Table I-2-2. Other Surface Structures of the Bacterial Cell

Pilus or fimbria 1. Common 2. Sex 3. Virulence	Primarily Gram – *	Glycoprotein (pilin)	Adherence to cell surfaces, including attachment to other bacteria during conjugation
Flagellum	+ and –	Protein (flagellin)	Motility
Axial filaments (internal flagellum)	Spirochetes Gram –	Protein	Motility

*M-protein of group A Strep described as diffuse fimbriate layer or fimbriae.

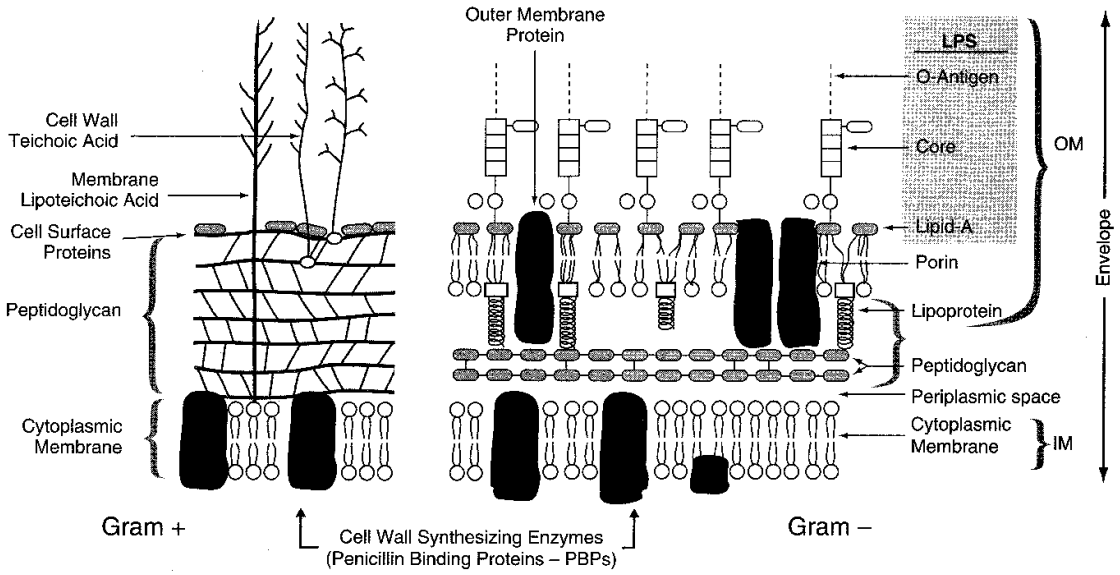


Figure I-2-2. Details of Cell Envelope and Peptidoglycan

STAINS

Table I-2-3. Gram Stain

Reagent	Gram-Positive	Gram-Negative
Crystal Violet (a very intense purple, small dye molecule)	Purple/Blue	Purple/Blue
Gram's Iodine	Purple/Blue (a large dye complex)	Purple/Blue (a large dye complex)
Acetone or Alcohol	Purple/Blue	Colorless
Safranin (a pale dye)	Purple/Blue	Red/Pink

All cocci are Gram-positive except *Neisseria* and *Moraxella*.
All spore formers are Gram-positive.
Background in stain modified for tissues will be pale red.

Table I-2-4. Ziehl-Neelsen Acid Fast Stain (or Kinyoun)

Reagent	Acid Fast	Non-Acid Fast*
Carbol Fuchsin with heat**	Red (Hot Pink)	Red (Hot Pink)
Acid Alcohol	Red	Colorless
Methylene Blue***	Red	Blue

* *Mycobacterium* is acid fast. *Nocardia* is partially acid fast. All other bacteria are non-acid fast except *Legionella micdadei*. Two protozoan parasites (*Cryptosporidium* and *Isospora*) have acid fast oocysts.
** Without the heat, the dye would not go in the mycobacterial cells.
*** Sputa and human cells will be blue.

Processing Sputa

In labs with fluorescent microscopes)

Screen Sputum Using Auramine-Rhodamine Fluorescent Stain

- Mycobacteria, *Nocardia*, and some other bacteria fluoresce a bright apple green on a black background.
- No antibody is involved.
- Test is sensitive (picks up high percent of AFB) but not very specific (picks up others, too).
- Negatives can be screened in 5 minutes (as opposed to 20 minutes for negative acid fast stains).

All positives are confirmed by acid fast stain.

Table I-2-5. Internal Bacterial Structures*

Structure	Cell Type	Chemical Composition	Function
Nucleoid region No membrane No histones No introns	Gram + and Gram –	DNA RNA Proteins	Genetic material (all essential genes) Primers, mRNA Linker proteins, polymerases
Plasmids	Gram + and Gram –	DNA	Non-essential genetic material Roles in conjugation, drug resistance, toxin production
Ribosomes	Gram + and Gram –	**70s** (protein/RNA) **30s** (16S RNA) **50s** (23 and 5s)	Protein synthesis
Granules (various types)	Gram + and Gram –	Glycogen, lipids, polyphosphate, etc.	Storage: polymerization of molecules present in high numbers in cells reduces osmotic pressure. Volutin granules of *Corynebacterium diphtheriae* are used in clinical identification.
Endospores	Gram + *only*	Keratin coat, calcium dipicolinate	Resistance to heat, chemicals, and dehydration

*Note that there are no mitochondria or membrane-bound structures like chloroplasts.

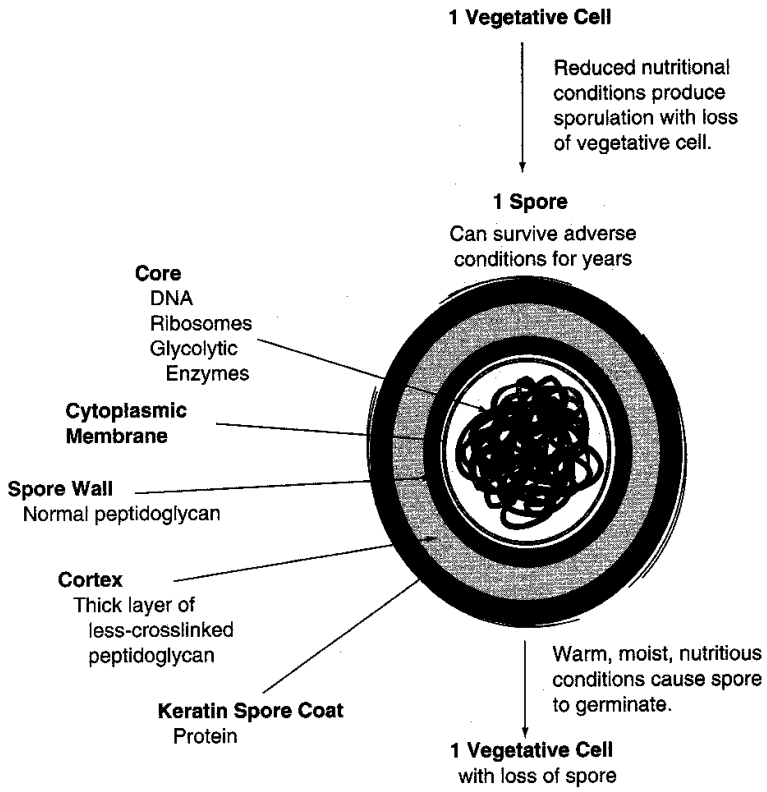


Figure I-2-3. Endospore

Note

Spores of fungi have a reproductive role.

ENDOSPORES

Organisms: *Bacillus* and *Clostridium*

Function

- Survival not reproductive (1 bacterium → 1 spore)
- Resistance to chemicals, dessiccation, radiation, freezing, and heat

Mechanism of Resistance

- New enzymes (i.e., dipicolinic acid synthetase, heat-resistant catalase)
- Increases or decreases in other enzymes
- Dehydration: calcium dipicolinate in core
- Keratin spore coat

BACTERIAL GROWTH AND DEATH

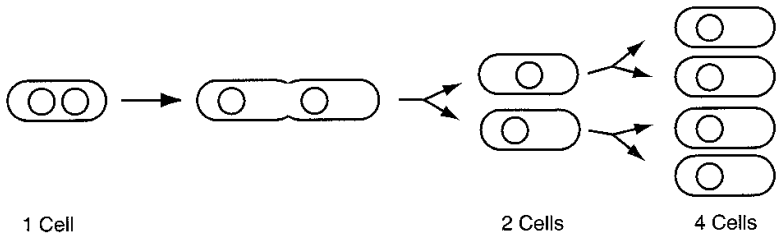


Figure I-2-4. Exponential Growth by Binary Fission

In A Nutshell

Lag Phase

- Initial Phase (only 1 lag phase)
- Detoxifying medium
- Turning on enzymes to utilize medium
- For exam, number of cells at beginning equals number of cells at end of lag phase.

Log Phase

- Rapid exponential growth
- Generation time = time it takes one cell to divide into two. This is determined during log phase.

Stationary Phase

- Nutrients used up
- Toxic products like acids and alkalis begin to accumulate.
- Number of new cells equals the number of dying cells.

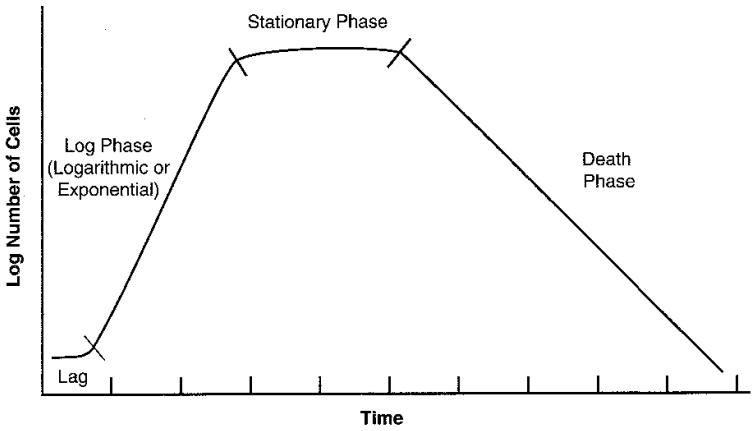


Figure I-2-5. Bacterial Growth Curve

Typical question:
A flask is inoculated to a density of 3×10^3 cells/ml. What is the density of cells in the culture after 50 minutes if the generation time is 20 minutes and the lag time is 10 minutes?

CULTURE OF MICROORGANISMS

- Obligate intracellular pathogens (viruses, rickettsias, chlamydias, etc.):
  Tissue cultures (cell cultures), eggs, animals, or not at all
- Facultative intracellular or extracellular organisms:
  Inert lab media (broths and agars)
  - Selective medium (S): A medium that selects for certain bacteria by inclusion of special nutrients and/or antibiotics.
  - Differential medium (D): A medium on which different bacteria can be distinguished by differences in colonial morphology or color.

Table I-2-6. Special Media for Selected Organisms

Organism	Medium
Anaerobes	**Thioglycolate**
Corynebacterium	Löffler's coagulated serum medium (S) Tellurite agar (D)
Enteric bacteria	Eosin methylene blue (D) MacConkeys (D)
Enteric pathogens	Hektoen enteric agar (D) Xylose-lysine-deoxycholate agar
Vibrio cholerae (likes alkaline growth medium)	TCBS (Thiosulfate Citrate Bile Salts Sucrose agar) (S)
Legionella	Charcoal-yeast extract agar (CYE agar) (S)
Mycobacterium	Löwenstein-Jensen medium (S)
Neisseria from normally sterile sites, *Haemophilus*	Chocolate agar
Neisseria from sites with normal flora	Thayer-Martin selective medium* (S)

*Thayer-Martin media is a chocolate agar supplemented with vancomycin, nystatin and colistin to inhibit the normal flora, including nonpathogenic *Neisseria*.

Table I-2-7. Miscellaneous Growth Requirements

Cholesterol and purines and pyrimidines	*Mycoplasma*
Cysteine*	*Francisella, Brucella, Legionella, Pasteurella*
X (protoporphyrin) and V (NAD)	*Haemophilus* (*influenzae* and *aegypticus* require both)

*The four Sisters Ella and the Cysteine Chapel.

ANAEROBIC AND AEROBIC

$$O_2^{\bullet -} + 2H^+ \xrightarrow{\text{superoxide dismutase}} H_2O_2 \xrightarrow{\text{catalase}} H_2O + 1/2\ O_2$$

Table I-2-8. Oxygen Requirements and Toxicity

Classification	Characteristics	Important Genera
Obligate aerobes	Require oxygen Have **no fermentative pathways** Generally produce superoxide dismutase	***Mycobacterium*** *Pseudomonas* (*Bacillus*)
Microaerophilic	Requires low but not full oxygen tension	*Campylobacter* *Helicobacter*
Facultative anaerobes	Will respire aerobically until oxygen is depleted and then ferment or respire anaerobically	Most bacteria, i.e., *Enterobacteriaceae*
Obligate anaerobes	1. Lack superoxide dismutase 2. Generally lack catalase 3. Are fermenters 4. Cannot use O_2 as terminal electron acceptor	*Actinomyces** *Bacteroides* *Clostridium*

*ABCs of anaerobiosis = *Actinomyces, Bacteroides,* and *Clostridium.*

Note

Mnemonic

The four sisters "Ella" worship in the "Cysteine" chapel:

- *Francisella*
- *Brucella*
- *Legionella*
- *Pasteurella*

BACTERIAL VACCINES

Childhood Vaccines

DTaP

- Totally **acellular**
- **Components of *B. pertussis:***
  - **Pertussis toxoid**
  - **± Filamentous hemagglutinin**
  - ± pertactin (adhesin)
- Diphtheria: **diphtheria toxoid**
  (= inactivated toxin that no longer causes disease but produces immunity)
- Tetanus: **tetanus toxoid**

In A Nutshell

Vaccine Efficacy

Neisseria vaccine

50% of cases are serotype B. It is not in the vaccine.

Haemophilus HIB vaccine

95% of cases are invasive disease caused by type B. The vaccine is against type B and is 95% effective

DTP

- Same D and T components as in DTaP, except the pertussis component is killed.

HIB (*Haemophilus influenzae* type b)

***H. influenzae* capsular polysaccharide conjugated to protein** (diphtheria toxoid or *Neisseria meningitidis* outer membrane proteins), making it a **T-cell–dependent vaccine** that infants respond to.

Senior Citizens or Asplenic

Streptococcus pneumoniae

Capsular polysaccharides of **23** different *Pneumococcus* strains

Limited Usage

Neisseria meningitidis

- **Four capsular polysaccharides:** Y, W-135, C, and A
- B serotype about 50% of cases in USA but capsule is sialic acid so not good immunogen.
- Used in outbreaks along with antibiotic prophylaxis
- Routine usage is in military recruits.

Salmonella typhi (ty21)

- **Attenuated bacterium** for travelers to endemic typhoid areas

Yersinia pestis

- **Killed cellular** vaccine (F-1 antigen)
- Military in endemic areas and *Y. pestis* laboratory workers

Bacillus anthracis

- **Supernatant** of partially purified proteins
- Military or occupational usage

BCG = Bacille Calmette Guérin (BCG)

- **Attenuated** (living) **strain of *Mycobacterium bovis***
- Doesn't prevent pulmonary tuberculosis but reduces dissemination

GRAM-STAINING REACTIONS

(†Marked organisms have high numbers of questions in the pool.)

Table I-2-9. Gram-Positive Bacteria

Cocci		
	Staphylococcus†	
	Streptococcus†	
Rods		
	Aerobic or facultative anaerobic	
		Bacillus
		Listeria
		Corynebacterium†
		Nocardia
		Mycobacterium†
	Anaerobic	
		Clostridium†
		Actinomyces
		Eubacterium
		Propionibacterium
		Lactobacillus

*Spore formers are *Bacillus* and *Clostridium*.

Table I-2-10. Non-Gram–staining Bacteria*

Mycoplasmataceae	
	Mycoplasma†
	Ureaplasma

*Note also:

Poorly visible on traditional Gram stain: ***Mycobacterium*** does not stain well with the Gram stain due to its waxy cell wall. It is considered Gram-positive.

Most **spirochetes**, **chlamydiae**, and **rickettsias** are so thin that the color of the Gram stain cannot be seen. All have Gram-negative cell walls.

Legionella (Gram-negative) also does not stain well with the traditional Gram stain unless counterstain time is increased.

Table I-2-11. Gram-Negative Bacteria

Aerobic		
	Cocci	*Neisseria*†
		Moraxella
	Rods	
		Pseudomonas
		Legionella
		Brucella
		Bordetella†
		Francisella
	Helical (and microaerophilic)	
		Campylobacter
		Helicobacter
Facultative anaerobic rods		
	Enterobacteriaceae†	
		Escherichia†
		Shigella
		Salmonella†
		Citrobacter
		Klebsiella
		Enterobacter
		Serratia
		Proteus
		Yersinia†
	Vibrionaceae	
		Vibrio
	Pasteurellaceae	
		Pasteurella
		Haemophilus†
Anaerobic straight to helical rods		
		Bacteroides/Prevotella
		Fusobacterium
	Spirochetes	
		Treponema†
		Borrelia
		Leptospira
	Rickettsiaceae and relatives	*Rickettsia*†
		Bartonella (Rochalimea)
		Coxiella
		Ehrlichia
	Chlamydiaceae	
		Chlamydia†

GENUS: *STAPHYLOCOCCUS*

- Gram-positive
- Cocci arranged in clusters
- Catalase positive

Table I-2-12. Medically Important Staphylococci*

Species	Coagulase/ Hemolysis	Additional Virulence Factors and Lab ID	Common Diseases
Staphylococcus aureus	Coagulase positive/ β-hemolysis	Protein A TSST-1 Enterotoxins Exfoliatins Cytolysins	Infective endocarditis (dominant cause in IV drug abusers) Abscesses Toxic shock syndrome Gastroenteritis Suppurative lesions Osteomyelitis
Staphylococcus epidermidis	Coagulase negative/ no hemolysis	Susceptible to novobiocin Normal skin flora Adherence: biofilm	Catheter & prosthetic device and infections Endocarditis in IV drug abusers
Staphylococcus saprophyticus	Coagulase negative/ no hemolysis	Resistant to novobiocin	Urinary tract infections in newly active adolescent women

*All members of the genus *Staphylococcus* are catalase positive.

Staphylococcus aureus

Distinguishing Characteristics

- **β-hemolytic**, yellow colonies of **Gram + cocci (clusters)** on blood agar (BA)
- **Catalase-positive, coagulase-positive**
- Salt tolerant; **ferments** mannitol **on mannitol salt agar**

Reservoir

- Normal flora on **nasal mucosa** and **skin.**

Transmission

- Spread via the **hands** and **sneezing**
- **Surgical wounds; lungs of cystic fibrosis patients**
- **Foods associated with food poisoning:**
  Ham or canned meats, custard pastries, and potato salad

Predisposing Factors for Infections

- **Surgery** or any break in skin, surgical packing or sutures or any foreign body (e.g., tampons); ventilators
- Severe **neutropenia** (<500/μL); cystic fibrosis
- **IV drug abuse** (IV drug abusers in general have more *S. aureus* on skin than *S. epidermidis*)
- **Chronic granulomatous disease** (CGD) Staph are catalase +

Pathogenesis

- Numerous enzymes and exotoxins
  - **Protein A inhibits phagocytosis;** binds Fc portion of antibody.
  - **Enterotoxins A-E** (heat stable 60°C, 10 min); fast 2–6 hours
  - **TSST-1** causes toxic shock syndrome. **Decreases** normal **liver clearance of endotoxin; superantigen** nonspecifically activates large numbers of T helper cells without processed antigen.
  - **Coagulases: convert fibrinogen to a fibrin clot**
  - **Cytolytic toxins**, e.g., **Staphylococcal alpha toxin**, a pore-forming toxin that damages human cell membranes
  - **Exfoliatins** involved in scalded skin syndrome (SSS) and formation of the bullae seen in staphylococcal impetigo

Table I-2-13. Staphylococcal Diseases

Diseases*	Clinical Symptoms	Pathogenicity Factors
Gastroenteritis (food poisoning) - toxin ingested preformed in food	2–6 hours after ingesting toxin: nausea, abdominal pain, vomiting, followed by diarrhea	Enterotoxins A–E preformed in food
Infective endocarditis	Fever, malaise, leukocytosis, heart murmur (may be absent initially)	Fibrin-platelet mesh, cytolytic toxins
Abscesses and mastitis	Subcutaneous tenderness, redness and swelling; hot	Coagulase, probably the cytolysins
Toxic shock syndrome	Fever, hypotension, scarlatiniform rash which desquamates (particularly on palms and soles), multiorgan failure	TSST-1
Impetigo	Erythematous papules to bullae	Coagulase, exfoliatins
Pneumonia	Productive pneumonia with rapid onset, high rate of necrosis and high fatality; nosocomial, ventilator, post-influenza, IV drug abuse, CF, CGD*, etc.	All
Surgical infections	Fever with cellulitis and/or abscesses	Coagulase, exfoliatins, ± TSSTs
Osteomyelitis	Bone pain, fever, ± tissue swelling, redness; lytic bone lesions on imaging	Cytolysins, coagulase

CF = cystic fibrosis; CGD = chronic granulomatous disease
*All except gastroenteritis may have scalded skin syndrome associated with the infection, especially in babies and the elderly.

Treatment

- Early on *S. aureus* (now known as methicillin-sensitive *S. aureus,* **MSSA**) acquired a multiple drug resistant plasmid with resistance to early beta-lactams (via a beta-lactamase) and most other antibiotics.
- Methicillin (and nafcillin) were developed.
- Methicillin-resistant *S. aureus* (**MRSA**) (due to changes in major penicillin-binding proteins) is **commonly resistant to all antibiotics except vancomycin** and **fusidic acid.**
- Topical mupirocin used to reduce nasal colonization

Prevention

- Basic hospital infection control

GENUS: *STREPTOCOCCUS*

- Gram-positive
- Chains or pairs of cocci
- Catalase negative

Hemolysis varies by species: Beta = clear; alpha = partial (green); gamma = non-hemolytic.

Streptococci are **serogrouped** using known antibodies to the **cell wall carbohydrates** (Lancefield's Groups A-O)

Streptococci are **serotyped** using known antibodies to the

- **Capsules** for ***Streptococcus pneumoniae***
- **M-protein for *Streptococcus pyogenes***

Table I-2-14. Medically Important Streptococci[1]

Species	Lancefield Group	Typical Hemolysis	Important Lab Characteristics
S. pyogenes	A	beta	Bacitracin-sensitive PYR test[2] positive
S. agalactiae	B	beta	Bacitracin-resistant Hippurate utilized CAMP test positive
Enterococcus faecalis[3]	D	alpha, beta or none	Growth in 6.5% NaCl PYR test positive
S. bovis[4]	D	alpha or none	No growth in 6.5% NaCl
S. pneumoniae	Not groupable[5]	alpha	Bile-soluble Inhibited by optochin
Viridans group	Not groupable	alpha	Not bile-soluble Not inhibited by optochin

[1] All streptococci are catalase negative.

[2] PYR test demonstrates the presence of pyrrolidonyl arylamidase. *S. pyogenes* is the one beta hemolytic *Streptococcus* that is positive; enterococci are positive.

[3] *Enterococcus faecalis* = *Streptococcus faecalis*. The enterococci belong to the Streptococcaceae family.

[4] *S. bovis* is a non-enterococcal group D organism.

[5] Not serogrouped because they lack the carbohydrate cell wall antigens.

Streptococcus pyogenes (Group A Streptococcus)

Distinguishing Characteristics

- Beta-hemolytic colonies inhibited by bacitracin on BA
- Gram positive cocci in chains, catalase-negative, PYR+

Reservoir

Human throat and skin

Transmission

Spread by respiratory droplets or direct contact

In a Nutshell

S. pyogenes

- group A
- beta hemolytic
- bacitracin sensitive

Pathogenesis

- **Hyaluronic acid capsule** (a polysaccharide) is **non-immunogenic**; inhibits phagocytic uptake.
- **M-protein**: virulence factor, **antiphagocytic, used to TYPE group A Strep; M12 strains**-associated with **acute glomerulonephritis**

Toxins

- **Streptolysin O: immunogenic**, hemolysin/cytolysin
- **Streptolysin S**: not immunogenic, hemolysin/cytolysin

Spreading factors:

- **Streptokinase**: breaks down fibrin clot
- **Streptococcal DNAse**: liquefies pus, extension of lesion
- **Hyaluronidase**: hydrolyzes the ground substances of the connective tissues; important to spread in cellulitis

Exotoxins A–C

(Pyrogenic or erythrogenic exotoxins.)

- **Phage-coded** (i.e., the cells are lysogenized by a phage.)
- Cause **fever and the rash** of Scarlet fever
- **Inhibit liver clearance of endotoxin** (from normal flora), creating shock-like conditions
- **Superantigens**: activate many helper T cells by bridging T cell receptors and MHC class II markers without processed antigen

Diseases

- *Streptococcus pyogenes* causes a wide variety of acute infections; some have immunologic sequelae.

Table I-2-15. Acute (Suppurative) *Streptococcus Pyogenes* Infections

Disease	Clinical Symptoms (Sx)
Pharyngitis	Abrupt onset of sore throat, fever, malaise and headache; tonsillar abscesses and tender anterior cervical lymph nodes
Scarlet fever	Above followed by a blanching, "sandpaper" rash; circumoral pallor; palms and soles are generally spared; strawberry tongue; nausea/vomiting
Pyoderma/ impetigo	Pus-producing skin infection (honey-crusted lesions)

Also, cellulitis/necrotizing fasciitis (flesh-eating bacteria!), puerperal fever, lymphangitis, pneumonia, a toxic shock-like syndrome, etc.

Table I-2-16. Non-suppurative Sequelae to Group A Streptococcal Infections

Disease	Sequelae to	Mechanism/Symptoms (Sx)
Rheumatic fever	Pharyngitis with Group A strep (not Group C)	Antibodies to heart tissue; mean = 19 d: fever, joint inflammation, carditis, erythema marginatum (chorea later) (Type II)
Acute glomerulonephritis (M12 serotype)	Pharyngitis or cutaneous	Immune complexes bound to glomeruli/ pulmonary edema and hypertension, dark urine (Type III)

Lab Notes

- For Strep throat: **rapid antigen test** (misses about 25% of the Strep throats); culture all "negatives."
- **ASO titer for rheumatic fever (>200 is significant)**

Treatment

- Beta-lactam drugs or erythromycin

Prevention

- Penicillin in rheumatic fever patients to prevent recurrent *S. pyogenes* pharyngitis

Streptococcus agalactiae = Group B Streptococci (GBS)

Distinguishing Characteristics

- **Beta-hemolytic,** bacitracin-resistant on BA
- **Gram-positive cocci in chains**
- **Group B** (determined by antiserum against cell wall carbohydrate in precipitin test)
- Catalase-negative, **hydrolyze hippurate**
- **CAMP test-positive: CAMP factor is a polypeptide** that "compliments" a *Staph aureus* sphingomyelinase to make an area of new complete beta-hemolysis.

Reservoir

- Colonizes human vagina (15–20% of women)

Transmission

- Newborn infected during birth
- Increased risk with **prolonged rupture of membranes**

Pathogenesis

- **Capsule**
- Beta-**hemolysin** and CAMP factor (an incomplete hemolysin)

Diseases

Neonatal septicemia and meningitis

- Group B Strep is the most common causative agent.

Treatment

- Ampicillin with cefotaxime or gentamicin

Prevention

- Treat mother prior to delivery if she has had a previous baby with GBS, has documented GBS colonization, or prolonged rupture of membranes.

In a Nutshell

S. agalactiae

- group B
- beta hemolytic
- bacitracin resistant
- hydrolyzes hippurate

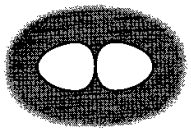


Figure I-2-6.
Streptococcus pneumoniae

In a Nutshell

S. pneumoniae

- alpha hemolytic
- bile soluble
- optochin sensitive

In A Nutshell

Typical Pneumonia

Bacterial pneumonia such as *Streptococcus pneumoniae* elicits neutrophils; arachidonic acid metabolites (acute inflammatory mediators) cause pain and fever. *Pneumococcus* produces a lobar pneumonia with a productive cough, grows on blood agar, and usually responds well to penicillin treatment.

Streptococcus pneumoniae (Pneumococcus)

Distinguishing Characteristics

- **Alpha-hemolytic** colonies **inhibited by optochin** on BA
- **Gram-positive, lancet-shaped diplococci** (or short chains)
- **Lysed by bile**

Reservoir

Human upper respiratory tract

Transmission

- **Respiratory droplets;** not considered highly communicable
- Often colonizes without causing disease.

Predisposing Conditions for Pneumonia

- Antecedent influenza or measles infection: damage to mucociliary elevator
- Chronic obstructive pulmonary disorders
- Congestive heart failure
- Alcoholism
- Asplenia predisposes to septicemia

Pathogenesis

- IgA protease: colonization
- Teichoic acids: attachment
- **Polysaccharide capsule: major virulence factor; retards phagocytosis** through inhibition of antibody-independent opsonization via the alternative complement pathway.
- **Quellung reaction** positive (swelling of capsule with type-specific antiserum)
- **Latex particle agglutination** test for capsular antigen in spinal fluid diagnostic for meningitis
- **Antibody** to the capsule (>80 serotypes) **provides type-specific immunity.**
- **Pneumolysin O:** hemolysin/cytolysin
  - **Damages respiratory epithelium** (hemolysin similar to streptolysin O, which damages eukaryotic cells).
  - (Inhibits leukocyte respiratory burst and inhibits classical complement fixation.)
- **Pneumococcus in alveoli** stimulate release of fluid and red and white cells producing "**rusty sputum.**"
- **Peptidoglycan/teichoic acids highly inflammatory** in CNS.

Diseases

Bacterial Pneumonia

- Most common bacterial cause, especially after 65 years but also in infants
- Sx: "big" shaking chill, high fever, lobar with productive blood-tinged sputum

Adult Meningitis
Most common cause. CSF generally has very high white cell count, low glucose.

Otitis Media and Sinusitis in Children
Most common cause

Treatment

- Penicillin G
- Resistance (both low level and high level) is chromosomal (altered penicillin-binding proteins); major concern in meningitis. (vancomycin ± rifampin used.)

Prevention

Vaccine 23 serotypes of capsule

Viridans Streptococci (*S. sanguis*, *S. mutans*, etc.)

Distinguishing Characteristics

- **Alpha-hemolytic, resistant to optochin**
- Gram-positive cocci in chains

Reservoir

Human **oropharynx (normal flora)**

Diseases

Dental Caries
S. mutans dextran-mediated adherence glues oral flora onto teeth, forming plaque and causing caries.

Infective Endocarditis

- Sx: malaise, fatigue, anorexia, night sweats, weight loss
- Predisposing conditions: damaged (or prosthetic) heart valve and dental work without prophylactic antibiotics **or** extremely poor oral hygiene

Pathogenesis

Dextran (biofilm)-mediated adherence onto tooth enamel or damaged heart valve and to each other (vegetation). Growth in vegetation protects organism from immune system.

Treatment

Penicillin G with aminoglycoside for endocarditis

Prevention

For individuals with damaged heart valve: prophylactic penicillin prior to dental work

In a Nutshell

Viridans streptococci

- alpha hemolytic
- bile resistant
- optochin resistant

GENUS: *ENTEROCOCCUS*

- **Catalase negative**
- **PYR+**

In a Nutshell

E. faecalis

- group D
- bile esculin positive
- grows in 6.5% salt

Enterococcus faecalis

Distinguishing Characteristics

- **Group D Gram-positive cocci in chains**
- **PYR test +**
- Catalase-negative, varied hemolysis
- **Hydrolyzes esculin in 40% bile and 6.5% NaCl** (bile esculin agar turns black)

Reservoir

Human colon, urethra ± and female genital tract

Pathogenesis/ Predisposing Conditions

- Bile/salt tolerance allows survival in bowel and gall bladder.
- During medical procedures on GI or GU tract: ***E. faecalis*** → **bloodstream → previously damaged heart valves → endocarditis**

Diseases

Urinary, biliary tract infections

Infective (subacute) endocarditis in persons (often elderly) with damaged heart valves

Treatment

- All strains carry some drug resistance.
- Some **vancomycin-resistant strains of *Enterococcus faecium*** or ***E. faecalis:*** no reliably effective treatment.
- (VanA strains have UDP-N-acetylmuramyl pentapeptide with the terminal D-alanyl-D-alanine replaced with D-alanyl-D-lactate, which functions in cell wall synthesis but is not blocked by vancomycin.)

Prevention

Prophylactic use of penicillin and gentamicin in patients with damaged heart valves prior to intestinal or urinary tract manipulations

Gram-Positive Rods

Table I-2-17. Medically Important Gram-positive Rods

	Spore Formation	Aerobic Growth	Motility	Exotoxin	Intracellular Growth	IC Host?***
Bacillus	+	+	+*	+	–	No
Clostridium	+	–	+**	+	–	No
Coryne-bacterium	–	+	–	+	–	No
Listeria	–	+	+ tumbling, actin "jetting"	–	+	Yes

* Most *Bacillus* spp. are motile (except *Bacillus anthracis*, which is nonmotile).
** Most clostridia are motile (except for *Clostridium perfringens*, which is nonmotile).
*** Is organism a significant problem in immunocompromised (IC) hosts?

GENUS: *BACILLUS*

- **Gram-positive rods**
- **Spore-forming**
- **Aerobic**

Bacillus anthracis

Distinguishing Characteristics

- **Large**, boxcar-like, Gram-positive, **spore-forming rods**
- **Capsule is polypeptide (poly-D-glutamate)** and the only non-polysaccharide one. It is immunogenic.

Reservoir

Animals, skins, soil

Transmission

Contact with infected animals or inhalation of spores from animal hair and wool. Spores survive long after animal dies.

Pathogenesis

Capsule: Polypeptide, antiphagocytic

Anthrax toxin
Includes three protein components:

- **Protective antigen** (B component)—mediates entry of LF or EF into eukaryotic cells
- **Lethal factor**—kills cells
- **Edema factor** is an adenylate cyclase (calmodulin-activated like pertussis adenylate cyclase).

Disease

Anthrax (rare in humans)

Cutaneous anthrax: Papule → papule with vesicles (malignant pustules) → central necrosis (eschar) with erythematous border often with painful regional lymphadenopathy; fever in 50%

Pulmonary (Wool Sorter's Disease): Life-threatening **pneumonia**; cough, fever, malaise, and ultimately facial edema, dyspnea, diaphoresis, cyanosis, and shock with **mediastinal hemorrhagic lymphadenitis**

Treatment

Ciprofloxacin or doxycycline

Prevention

Vaccine: cell free vaccine for people in high-risk occupations

Bacillus cereus

- **Spores** found widely in nature, including food, and are not killed by boiling.
- **Food poisoning** associated with food held warm (not hot)
- Two possible toxins:
  - **Emetic toxin: fast (1–6 hours)**, similar to *S. aureus* with vomiting and diarrhea; associated with **fried rice**
  - Diarrheal toxin (meats, sauces): 18 hours, similar to *E. coli*; LT: increasing cAMP → watery diarrhea

GENUS: *LISTERIA*

- **Gram-positive, nonspore-forming rods**
- **Facultative intracellular**
- **Tumbling motility**

Listeria monocytogenes

Distinguishing Characteristics

- Small Gram-positive rods
- Beta hemolytic, nonspore-forming rod on BA
- **Tumbling motility** in broth; actin jet motility in cells
- **Facultative intracellular parasite**
- **Cold growth**

Reservoir

- Widespread: animals (gastrointestinal and genital tracts), **unpasteurized milk products,** plants, and soil
- Cold growth: soft cheeses, deli meats, cabbages (coleslaw)

Transmission

Foodborne, across the placenta, or by contact during delivery

Pathogenesis

- **Listeriolysin O, a β-hemolysin:** facilitates rapid egress from phagosome into cytoplasm, thus evading killing when lysosomal contents are dumped into phagosome; "jets" directly (by actin filament formation) from cytoplasm to another cell.
- **Immunologic immaturity predisposes to serious infection.**

Diseases

Listeriosis (human, peaks in summer)

- Healthy adults and children: generally asymptomatic or diarrhea with low % carriage
- Pregnant women: symptomatic carriage, septicemia characterized by fever and chills; can cross the placenta in septicemia.

Neonatal Disease

Early-onset: (granulomatosis infantiseptica) *in utero* transmission; sepsis with high mortality; disseminated granulomas with central necrosis.

Late-onset: 2–3 weeks after birth from fecal exposure; meningitis with septicemia.

Immunocompromised Patients (IC pts)

- **Septicemia and meningitis** (most common clinical presentation)
- *Listeria* meningitis is the most common cause of meningitis in **renal transplant patients and adults with cancer.**

Treatment

Ampicillin with gentamicin added for IC patients

Prevention

Precautions with food may reduce incidence.

GENUS: *CORYNEBACTERIUM*

- **Gram-positive rods**
- **Nonspore-forming, nonmotile**
- **Aerobic**

Corynebacterium diphtheriae

Distinguishing Characteristics

- Gray to black colonies of **club-shaped** Gram-positive rods arranged in V or L shapes on **tellurite medium**.
- **Granules (volutin)** produced on Loeffler's coagulated serum medium stain metachromatically
- Aerobic, nonspore-forming
- **Toxin-producing strains have β-prophage** carrying genes for the toxin (**lysogeny, β-corynephage**). The phage from one patient with diphtheria can infect the normal nontoxigenic diphtheroid of another person and cause diphtheria.

Transmission

Bacterium or phage via respiratory droplets from oropharynx of infected person

Pathogenesis

Organism **not invasive;** colonizes epithelium of oropharynx or skin in cutaneous diphtheria.

Diphtheria toxin (A-B component)—inhibits protein synthesis by adding ADP-ribose to EF-2.

- Effect on oropharynx:
  - **Dirty gray pseudomembrane** (made up of dead cells and fibrin exudate, bacterial pigment)
  - **Extension into larynx/trachea → obstruction**
- Effect of systemic circulation → **heart** & **nerve** damage

Disease

Diphtheria

Sore throat with **pseudomembrane, bull neck**, potential respiratory obstruction, **myocarditis,** cardiac dysfunction, **recurrent laryngeal nerve palsy,** and lower limb polyneuritis.

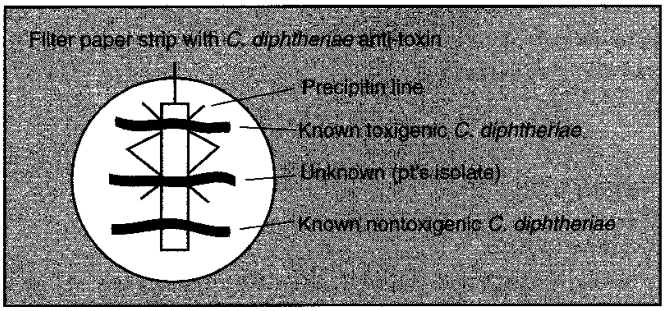

Filter paper strip with C. diphtheriae anti-toxin
Precipitin line
Known toxigenic C. diphtheriae
Unknown (pt's isolate)
Known nontoxigenic C. diphtheriae

Figure I-2-7. Elek Test

Lab Notes

- Elek test to document toxin production.
- Toxin produced by Tox+ strains diffuses away from growth.
- Antitoxin diffuses away from the strip of filter paper.
- Precipitin lines form at zone of equivalence.

Treatment

Erythromycin and **antitoxin**

Prevention

Toxoid vaccine (formaldehyde-modified toxin is still immunogenic but with reduced toxicity), part of DTaP, DTP, or Td

GENUS: *ACTINOMYCES*

- **Anaerobic BACTERIA**
- **Gram-positive rods to branching filaments**
- **Not acid fast**

Actinomyces israelii

Distinguishing Characteristics

Anaerobic, Gram-positive branching rods

Reservoir

Human; normal flora of **gingival crevices** and **female genital tract**

Transmission

Endogenous

Pathogenesis

Invasive growth in tissues with compromised oxygen supply; anaerobic growth

Disease

Actinomycosis

- Generally not painful but **very invasive,** penetrating all tissues including bone
- **Tissue swelling → draining abscesses** (sinus tracts) with **"sulfur" granules** (hard yellow microcolonies) in exudate that can be used for microscopy or culture
- Only in tissues with low oxygenation (E_h)

Forms

- **Cervicofacial (lumpy jaw): dental trauma or poor oral hygiene**
- **Pelvic: from thoracic or sometimes IUD's**
- Thoracic: aspiration with contiguous spread
- Abdominal: surgery or bowel trauma
- CNS: **solitary brain abscess** most common

Treatment

Ampicillin or **penicillin G** and **surgical drainage**

In a Nutshell

Actinomyces

- Gram +
- Not acid fast
- Anaerobic
- Endogenous infection
- Penicillin

GENUS: *NOCARDIA*

- BACTERIA
- **Gram-positive filaments breaking up into rods**
- **Aerobic**
- **Partially acid-fast (some areas of smear will be blue and some red)**

Nocardia asteroides

Distinguishing Characteristics

- **Aerobic**
- **Gram-positive branching rods**
- **Partially acid-fast**

In A Nutshell

Nocardia

- Gram +
- Partially acid fast
- Aerobic
- Exogenous
- Sulfa drugs

Reservoir

Soil, dust

Transmission

Airborne or traumatic implantation

Pathogenesis

- No toxins or virulence factors known
- **Immunosuppression and cancer predispose** to pulmonary infection.

Diseases

Nocardiosis

- **Cavitary bronchopulmonary nocardiosis**
  - Sx: cough, fever, dyspnea
  - May spread hematogenously to brain (**brain abscesses**)
- **Cutaneous/subcutaneous nocardiosis**
  - Starts with traumatic implantation
  - Sx: **cellulitis** with swelling → **draining subcutaneous abscesses** with **granules** (mycetoma)

Treatment

Sulfonamides (high dose) or trimethoprim/sulfamethoxazole

Table I-2-18. Mycobacteria and Close Relatives

Genus	Aerobic Growth?	Acid Fast?	Morphology (Don't memorize!)
Corynebacterium	Yes	Not AF	Rods
Actinomyces	No, anaerobe	Not AF	Rods, filaments
Nocardia	Yes	Partially AF	Rods, filaments
Mycobacterium	Yes, obligate aerobe	Yes, AF	Rods

GENUS: *MYCOBACTERIUM*

- **Acid fast rods with waxy cell wall**
- **Obligate aerobe**

Cell Wall

- Unique: **high concentration of lipids** containing long chain fatty acids called mycolic acids
- Structural organization shown in Figure II-8.

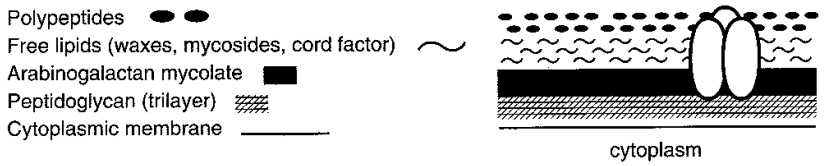


Figure I-2-8. Mycobacterial Cell Wall

- Wall makes **mycobacteria highly resistant to**
  - **Desiccation**
  - **Many chemicals** (including NaOH used to kill other bacteria in sputa before neutralizing and culturing)
- **Mycobacteria are sensitive to UV**

Mycobacterium tuberculosis

Distinguishing Characteristics

- **Auramine-rhodamine staining bacilli** (fluorescent apple green); no antibody involved (sensitive but not specific)
- **Acid fast**
- **Aerobic, slow growing** on **Lowenstein-Jensen** medium; new culture systems (broths with palmitic acid) faster
- **Produces niacin**

- Produces a **heat-sensitive catalase:**
  - Catalase negative at 68°C (standard catalase test)—(other mycobacterial catalases are heat insensitive)
  - Catalase active at body temperature.

Reservoir

Human lungs

Transmission

Respiratory droplets and **droplet nuclei**

Predisposing Factor

For **active disease is poverty, HIV infection, or any CMI system immunosuppression.**

Pathogenesis

Facultative Intracellular Organism
Sulfatides (**sulfolipids** in cell envelope)

- Inhibit the phagosome-lysosomal fusion allowing intracellular survival. (If fusion occurs, waxy nature of cell envelope reduces killing effect.)

Cord factor (trehalose di-mycolate)

Figure I-2-9.
Cord Factor

- Causes **serpentine growth** *in vitro*
- **Inhibits leukocyte migration; disrupts mitochondrial respiration and oxidative phosphorylation**

Tuberculin (surface protein) along with mycolic acid → delayed hypersensitivity and CMI

- Granulomas and caseation mediated by **cell-mediated immunity** (CMI)
- No exotoxins nor endotoxin; damage done by immune system

Disease

Tuberculosis

- Causative agents: *Mycobacterium tuberculosis* and *M. bovis*
- Complex disease: pulmonary, urinary tract, any organ or miliary (disseminated) (see pathology notes).
- Primary infection: organisms replicate in naïve macrophages, killing macrophages until CMI is set up.
- Most people heal without disease; **some organisms walled off in the Ghon complex remain viable unless treated.**
- Post primary (reactivational TB) erosion of granulomas into airways (high oxygen) later in life under conditions of reduced T-cell immunity leads to mycobacterial replication and disease symptoms.

Diagnosis

- Microscopy on specimen: auramine-rhodamine stain (fluorescent apple green); no antibody involved; very sensitive; if positive followed by acid fast stain.
- **PPD skin test** (Mantoux): measure **zone of induration at 48–72 hours; positive if zone of induration at 48–72 hours is:**

  ≥5 mm in HIV+ or anyone with recent TB exposure; AIDS patients have reduced ability to mount skin test.

  ≥10 mm in high-risk population: IV drug abusers, people living in poverty, or immigrants from high TB area.

  ≥15 mm in low-risk population.
- **Positive skin test indicates** only **exposure but not necessarily active disease.**
- Slow-growing (3–6 weeks) colony on Lowenstein-Jensen medium (faster new systems)
- Organisms produce **niacin** and are **catalase-negative** (68°C).
- **No serodiagnosis**

Treatment

- **Multiple drugs critical** to treat infection
- Standard observed short-term therapy for uncomplicated pulmonary TB (rate where acquired <4%):
  - First 2 months: isoniazid + rifampin + pyrazinamide
  - Next 4 months: isoniazid and rifampin
- Ethambutol or streptomycin added for possible drug-resistant cases until susceptibility tests are back (if area acquired has >4% DRM TB)

Prevention

- **Isoniazid** taken for 6–9 months can prevent TB in persons with infection but no clinical symptoms.
- Bacille-Calmette-Guérin (BCG) **vaccine** containing live, attenuated organisms may prevent disseminated disease. Not commonly used in the U.S.
- UV lights or HEPA filters used to treat potentially contaminated air.

Mycobacteria Other Than Tuberculosis (MOTTS)

- (**MOTTS**) = Non-tuberculous Mycobacteria = atypical **Mycobacteria**
- **Non-contagious!**
- Found in **surface waters, soil,** cigarettes; most common in southeastern U.S.
- Runyon terminology still used, particularly for pigmented ones. Dr. Runyon grew two tubes of each in the dark and then exposed one of the pair to bright light for 2 hours after the cultures were grown up.

Table I-2-19. Runyon Grouping of MOTTS

Species*	Runyon Grouping	Pigment in Dark**	Pigment in Light	Growth
M. kansasii	**Photochromogen**	–	+	slow
M. scrofulaceum	Scotochromogens	+	+	slow
M. avium-intracellulare	**Non-chromogens**	–	–	slow

* Not classified: *M. tuberculosis*, *M. bovis*, and *M. ulcerans*. None produce pigments. Also not included is *M. leprae*, which cannot be grown in the lab.
** Pigments are carotenoids.
Note: Speciation now uses biochemical methods; some strains of *M. avium-intracellulare* complex (MAI or MAC) may produce pale yellow pigments.

Diseases

Pulmonary/Gastrointestinal/Disseminated

- Patients: **AIDS** (prophylaxis < 75 CD4+ cells/mm^3), **cancer, chronic lung disease**
- ***M. avium-intracellulare, M. kansasii.***

Mycobacterial lymphadenitis

- Usually solitary cervical lymph nodes (surgically removed) in kids.
- *M. scrofulaceum.*

Soft-Tissue Infections

M. marinum: cutaneous granulomas in tropical fish enthusiasts (fish tank granuloma) or scuba divers from abrasions on coral

Note

M. tuberculosis with normal CD4 count or with low CD4 count (disseminated).

MAI only late with low CD4 count.

Mycobacterium leprae

Distinguishing Characteristics

- **Acid fast rods (seen in punch biopsy)**
- **Obligate intracellular parasite** (cannot be cultured *in vitro*)
- Optimal growth at less than body temperature

Reservoir

- **Human** mucosa, skin, and nerves are the only significant reservoir.
- Some infected armadillos in Texas and Louisiana

Transmission

Nasal discharge from untreated lepromatous leprosy patients

Pathogenesis

- **Obligate intracellular parasite**
- Cooler parts of body, e.g., skin, mucous membranes, and peripheral nerves

Disease

Leprosy: A continuum of disease, which usually starts out with an indeterminate stage called "borderline."

Table I-2-20. Two Extreme Forms of Leprosy

	Tuberculoid	Borderline	Lepromatous
Cell-mediated immune system	Strong CMI		Weak CMI
Lepromin skin test	Lepromin test +		Lepromin test –
Number of organisms in tissue	Low		High (foam cells totally filled)
Damage from	Immune response (CMI killing infected cells) Granuloma formation → nerve enlargement/damage Loss of sensation → burns and trauma		Large number of intracellular organisms Nerve damage from overgrowth of bacteria in cells Loss of sensation → burns and trauma
Number of lesions and other symptoms	Fewer lesions: macular; nerve enlargement, paresthesia		Numerous lesions becoming nodular; loss of eyebrows; destruction of nasal septum Paresthesia Leonine facies

Laboratory Diagnosis

- **Punch biopsy or nasal scrapings; acid fast stain**
- **Lepromin skin test** is positive in the tuberculoid but not in the lepromatous form.
- (No cultures)

Treatment

Multiple-drug therapy with dapsone and rifampin, with clofazimine added for lepromatous

Prevention

Dapsone for close family contacts

GENUS: *CLOSTRIDIUM*

- **Gram-positive rod**
- **Spore-forming**
- **Anaerobic**

Clostridium tetani

Distinguishing Characteristics

- **Large Gram-positive, spore-forming rods**
- Anaerobes
- Produces tetanus toxin

Reservoir

Soil

Transmission

- Puncture wounds/trauma
- Requires low tissue oxygenation (E_h)

Disease

Tetanus; sx: risus sardonicus, opisthotonus

Pathogenesis

Spores germinate in the tissues producing **tetanus toxin** (an exotoxin also called **tetanospasmin**).

- Carried intra-axonally to CNS
- **Binds to ganglioside receptors**
- **Blocks release of inhibitory mediators (glycine and GABA) at spinal synapses**
- Excitatory neurons are unopposed → extreme muscle spasm
- One of the most toxic substances known

Diagnosis

Primarily a clinical diagnosis, organism is rarely isolated

Treatment

Of actual tetanus:

- **Hyperimmune human globulin** (TIG) **to neutralize toxin, + metronidazole or penicillin**
- **Spasmolytic drugs** (diazepam), debride, delay closure

Prevention

Toxoid Vaccines (DTP, DTaP, Td)

- Toxoid is formaldehyde-inactivated toxin.
- Important because disinfectants have poor sporucidal action
- Care of wounds: proper wound cleansing and care plus treatment

Table I-2-21. Wound Management

	Not Tetanus Prone linear, 1 cm deep cut, without devitalized tissue, without major contaminants, less than 6 hours old	**Tetanus Prone** blunt/missile, burn, frostbite, 1 cm deep; devitalized tissue present + contaminants (e.g., dirt, saliva), any wound 6 hours old
Not completed primary or vaccination history unknown	Vaccine	Vaccine and TIG*
Completed primary series	Vaccine if more than 10 years since last booster	Vaccine if more than 5 years since last booster

* TIG = tetanus immunoglobulin (human).

Clostridium botulinum

Distinguishing Characteristics

- **Anaerobic, Gram-positive, spore-forming rods**

Reservoir

- Soil/dust

Pathogenesis

Spores survive in soil and dust; **germinate** in moist, warm, nutritious but **non-acidic and anaerobic conditions.**

Botulinum toxin

- **A-B polypeptide neurotoxin** (actually a series of 7 antigenically different; Type A and B most common)
- **Coded for by a prophage** (lysogenized *Clostridium botulinum*).
- Highly toxic
- **Heat labile** (unlike staph), 10 minutes 60°C
- **Mechanism of action:**
  - **Absorbed by gut** and carried by blood to peripheral nerve synapses
  - **Blocks release of acetylcholine** at the myoneuronal junction resulting in a reversible **flaccid paralysis**

Table I-2-22. Forms of Botulism

Disease	Adult/food borne	Infant	Wound
Acquisition	**Preformed toxin ingested (toxicosis)** Poorly canned alkaline vegetables (green beans) Smoked fish	**Spores ingested: household dust, HONEY** **Toxin produced in gut (toxi-infection)**	**Traumatic implantation** of spores; *in vivo* production of toxin **(toxi-infection)** Debridement, no closure
Symptoms	1–2 day onset of Sx: weakness, dizziness, blurred vision, flaccid paralysis (reversible); ±diarrhea, nausea or vomiting	Constipation, limpness/ flaccid paralysis (reversible): diplopia, dysphagia, weak feeding/crying; may lead to respiratory arrest	As for food without GI symptoms
Toxin demonstrated in	Suspected food or serum	Stool or serum	Serum
Treatment	Respiratory support Polyvalent antitoxin	Respiratory support in monitored intensive care; Hyperimmune human serum Antibiotics generally not used as may worsen or prolong	Rx: amoxicillin **and antitoxin (respiratory support)**
Prevention	**Proper canning; Heat all canned foods**	**No honey first year**	

Clostridium perfringens

Distinguishing Characteristics

- Large **Gram-positive, spore-forming** rods (spores rare in tissue), nonmotile
- **Anaerobic: "stormy fermentation"** in milk media
- **Double zone of beta hemolysis**

Reservoir

Soil and human colon

Pathogenesis

- **Spores** germinate under anaerobic conditions in tissue.
- Vegetative cells produce:

  Alpha toxin (a.k.a., phospholipase C) is a **lecithinase.** It disrupts membranes, damaging RBC, platelets, WBC, endothelial cells → massive hemolysis, tissue destruction, hepatic toxicity.
- Identified by **Naegler's reaction**: egg yolk agar plate—one side with anti-α-**toxin.** Lecithinase activity is detected on side with no antitoxin.
- 12 other toxins damage tissues.
- **Enterotoxin** produced in intestines in food poisoning: disrupts ion transport → watery diarrhea, cramps (similar to *E. coli*); resolution <24 hours.

Diseases

Gas Gangrene (Myonecrosis)

- Contamination of **wound with soil or feces**
- **Acute** and **increasing pain** at wound site
- **Tense tissue** (edema) and exudate
- Systemic symptoms include **fever** and **tachycardia** (disproportionate to fever), diaphoresis, pallor, etc.
- **Rapid, high mortality**
- Prevention: extensive **debridement** of the wound plus administration of penicillin decreases probability
- Treatment: debridement, delayed closure, clindamycin and penicillin, hyperbaric chamber

Food Poisoning

- **Reheated meat dishes**, organism grows to high numbers; 8–24 hour incubation
- **Enterotoxin** production in gut; self-limiting non-inflammatory, watery diarrhea
- Treatment: supportive for food poisoning

Clostridium difficile

Antibiotic-associated (clindamycin, cephalosporins, amoxicillin, ampicillin) **diarrhea, colitis, or pseudomembranous colitis** (yellow plaques on colon)

Two Toxins

Toxin A: Enterotoxin damaging mucosa leading to fluid increase; granulocyte attractant

Toxin B: Cytotoxin: cytopathic

Treatment

Metronidazole (vancomycin): use only if no other drug available; to avoid selecting for vancomycin-resistant normal flora.

GENUS: *NEISSERIA*

- **Gram-negative**
- **Diplococci with flattened sides**
- **Oxidase positive**

Note

Oxidase

Oxidase (cytochrome C oxidase) test: flood colony with phenylenediamine; in presence of oxidase, phenylenediamine turns black. Rapid test.

Major oxidase-negative Gram-negative group is Enterobacteriaceae.

Table I-2-23. Medically Important *Neisseria*

Species	Capsule	Vaccine	Portal of Entry	Glucose Utilization	Maltose Fermentation	β-lactamase Production
N. meningitidis	+	Yes	Respiratory	+	+	Rare
N. gonorrhoeae	–	No	Genital	+	–	>16%

Neisseria meningitidis (*Meningococcus*)

Distinguishing Characteristics

- **Gram-negative** kidney bean-shaped **diplococci**
- **Large capsule; latex particle agglutination** (or CIE; counter immunoelectrophoresis) **to identify *N. meningitidis* capsular antigens in CSF**
- Grows on **chocolate (not blood) agar in 5% CO_2** atmosphere
- **Ferments maltose** in contrast to gonococci

Reservoir

Human nasopharyngeal area

Transmission

- Respiratory droplets; oropharyngeal colonization, spread to the meninges via the bloodstream
- Disease occurs in only small percent of colonized.

Pathogenesis

Important Virulence Factors

- Polysaccharide **capsule**: antiphagocytic, antigenic, 5 common serogroups: B is not strongly immunogenic (sialic acid), B strain is most common strain in USA. Used for: serogrouping, detection in CSF, and vaccine
- **IgA protease** allows oropharynx colonization.
- **Endotoxin** (LPS): fever, septic shock in meningococcemia, **overproduction of outer membrane**
- Pili and outer membrane proteins important in ability to colonize and invade
- Deficiency in late complement components (C5-8) predisposes to bacteremia.

Disease

Meningitis and Meningococcemia

- **Abrupt onset with fever, chills, malaise, prostration, and a rash that is generally petechial**; rapid decline
- Fulminant cases: ecchymoses, DIC, shock, coma, and death (Waterhouse-Friderichsen syndrome)

Treatment

- Penicillin G or ceftriaxone
- Some β-lactamase production beginning to be seen

Prevention

- **Vaccine**: capsular polysaccharide of strains **Y, W-135, C, and A**
  - Type B (50% of the cases in USA) capsule not a good immunogen
- Prophylaxis of close contacts: **rifampin** (or ciprofloxacin)

Neisseria gonorrhoeae (*Gonococcus*)

Distinguishing Characteristics

- **Gram-negative** kidney bean-shaped **diplococci**
- (Intracellular **Gram (–) diplococci in PMNs** from urethral smear from symptomatic male is suggestive of *N.g.*)
- Commonly: diagnosis by **genetic probes** with amplification
- Culture (when done) on **Thayer-Martin medium**
  - Oxidase-positive colonies
  - Maltose not fermented
  - No capsule

Reservoir

Human genital tract

Transmission

Sexual contact, birth; sensitive to drying and cold.

Pathogenesis

Pili

- **Attachment** to mucosal surfaces
- **Inhibit phagocytic uptake**
- **Antigenic** (immunogenic) **variation**: >1 million variants

Outer Membrane Proteins

- OMP I: structural, antigen used in serotyping
- Opa proteins (opacity)**: antigenic variation**, adherence
- **IgA protease**: aids in colonization and cellular uptake

Organism invades mucosal surfaces and causes inflammation.

Disease

Gonorrhea

- Male: **urethritis**, proctitis
- Female: **endocervicitis**, PID (contiguous spread), arthritis, proctitis
- Infants: **ophthalmia** (rapidly leads to **blindness if untreated**)

Treatment

- Ceftriaxone
- Test for *Chlamydia trachomatis* or treat with a tetracycline
- Penicillin-binding protein mutations led to gradual increases in penicillin resistance from the 50s to the 70s.
- Plasmid-mediated **β-lactamase** produces **high-level penicillin resistance.**

Prevention

- Adult forms: no vaccine; condoms
- Neonatal: silver nitrate or erythromycin ointment in eyes at birth

Moraxella catarrhalis

- Gram-negative diplococcus (close relative of neisseriae)
- Normal upper respiratory flora
- **Otitis media**
- Causes bronchitis and bronchopneumonia in elderly with COPD
- Drug resistance a problem; most strains produce a **β-lactamase**

GENUS: *PSEUDOMONAS*

- **Gram-negative rod**
- **Oxidase-positive**
- **Aerobic**

Pseudomonas aeruginosa

Distinguishing Characteristics

- **Oxidase-positive, Gram-negative rods, non-fermenting**
- **Pigments: pyocyanin (blue-green)** and fluorescein
- **Grape-like odor**
- **Slime layer**
- **Non-lactose-fermenting** colonies on EMB or MacConkey

Reservoir

Ubiquitous in water

Transmission

Water aerosols, raw vegetables, flowers

Pathogenesis

- **Endotoxin** causes inflammation in tissues and Gram-negative shock in septicemia.
- ***Pseudomonas* exotoxin A ADP ribosylates EF-2**, inhibiting protein synthesis (like diphtheria toxin)
- **Liver is primary target.**
- **Capsule/slime layer**: allows formation of pulmonary **microcolonies;** difficult to remove by phagocytosis

Compromising Condition/Opportunistic Infections

Normal People

- Transient GI tract colonization: loose stools (10% pop.)
- Hot tub folliculitis
- Eye ulcers: trauma, coma, or prolonged contact wear

Burn Patients: GI tract colonization → skin → colonization of eschar → **cellulitis (blue-green pus)** → **septicemia**

Neutropenic Patients: Pneumonia and **septicemias**—often **superinfections** (infections while on antibiotics)

Chronic Granulomatous Disease (CGD): Pneumonias, septicemias (*Pseudomonas* is catalase positive)

Note

***Pseudomonas* medical ecology**

Pseudomonas aeruginosa is an **ubiquitous water** and soil organism that grows to very high numbers overnight in standing water (distilled or tap).

Sources for infections include:

- Raw vegetables, respirators, humidifiers, sink drains, faucet aerators, cut and potted flowers, and, if not properly maintained, whirlpools.
- Transient colonization of colons of about 10% of people. Bacteria get on skin from fecal organisms. Requires exquisitely careful housekeeping and restricted diets in burn units.

Septicemias: Fever, shock ± skin lesions (black necrotic center, erythematous margin (**ecthyma gangrenosum**)

Catheterized Patients: Urinary tract infections (UTI)

Cystic Fibrosis: Early pulmonary colonization, recurrent pneumonias. Always high **slime-producing strains**

Treatment

Antipseudomonal penicillin, third-generation cephalosporins

Prevention

- Pasteurization or disinfection of water-related equipment, hand washing; prompt removal of catheters.
- No flowers or raw vegetables in burn units.

Note

Drug Resistance in *P. aeruginosa*

Susceptibilities important.

Drug resistance very common:

Inherent resistance (missing high affinity porin some drugs enter through); Plasmid-mediated β-lactamases and acetylating enzymes.

GENUS: *LEGIONELLA*

- **Weakly Gram-negative**
- **Pleomorphic rods requiring cysteine and iron**
- **Water organisms**

Legionella pneumophila (and other legionellae)

Distinguishing Characteristics

- **Stain poorly** with standard Gram stain; **Gram-negative**
- **Fastidious** requiring increased **iron and cysteine** for laboratory culture (CYE, Charcoal Yeast Extract)
- **Facultative intracellular pathogens**
  - Diagnosis: **DFA** (Direct Fluorescent Antibody) on biopsy, (+) by Dieterle silver stain
  - Antigen urine test for serogroup 1 only
  - Fourfold increase in antibody

Reservoir

Rivers/streams/amoebae; air-conditioning water cooling tanks

Transmission

- Aerosols from contaminated **air-conditioning**
- **No human-to-human transmission**

Predisposing Factors

- **Smokers over 55 years with high alcohol intake**
- **Immunosuppressed patients**, e.g., renal transplant patients

Pathogenesis

- **Facultative intracellular** pathogen
- **Endotoxin**

Diseases

Seasonal: Associated with air-conditioning systems, now routinely decontaminated

Legionnaires' Disease ("Atypical Pneumonia")

- Pneumonia
- Mental confusion
- Diarrhea (no *Legionella* in gastrointestinal tract)

Pontiac Fever

- Pneumonitis
- No fatalities

Treatment

- Fluoroquinolone or azithromycin or erythromycin with rifampin for immunocompromised patients
- Drug must penetrate human cells.

Prevention

Routine decontamination of air-conditioner cooling tanks

GENUS: *BORDETELLA*

- **Gram-negative small rods**
- **Strict aerobes**

Bordetella pertussis

Distinguishing Characteristics

- Small **Gram-negative**, aerobic **rods**
- Fastidious/delicate: **Regan-Lowe** or Bordet-Gengou media; either direct cough plates or nasopharyngeal cultures.
- Difficult to culture from the middle of paroxysmal stage on
- Direct immunofluorescence (**DFA**) on nasopharyngeal smear
- PCR and serologic tests available
- Encapsulated organism

Reservoir/Transmission

Human (vaccinated); respiratory droplet

Pathogenesis

***B. pertussis* Is a Mucosal Surface Pathogen**
Attachment to nasopharyngeal ciliated epithelial cells

- Filamentous hemagglutinin
- Pertussis toxin (on outer membrane) aids in attachment

Toxins damage respiratory epithelium.

- **Adenylate cyclase toxin**: impairs leukocyte chemotaxis → inhibits phagocytosis and causes local edema
- **Tracheal cytotoxin**: interferes with ciliary action; kills ciliated cells
- Endotoxin
- **Pertussis toxin** (A and B component, OM protein toxin):
  ADP ribosylation of G_i (inhibiting negative regulator of adenyl cyclase) interferes with transfer of signals from cell surface to intracellular mediator system;
  - Lymphocytosis promotion
  - Islet-activation → hypoglycemia
  - Blocks immune effector cells
  - Increased histamine sensitivity

In A Nutshell

***B. pertussis* immunity**

- Vaccine immunity lasts 5–10 years (and is primarily IgA)
- Babies born with little immunity.
- Vaccinated humans >10 yrs serve as reservoir.
- 12–20% of afebrile adults with cough >2 weeks have pertussis.
- Immunity to actual pertussis is life long.
- New vaccines (DTaP)
  - Acellular: components:
    - Immunogens vary by manufacturer
    - Pertussis toxoid
    - Filamentous hemagglutinin
    - Pertactin (OMP)
    - 1 other

Disease

Whooping Cough (Pertussis)
Three stages after a 7–10 day incubation; contagious

- (1–2 weeks) **catarrhal:** rhinorrhea, malaise, fever, sneezing; contagious
- (2–4 weeks) **paroxysmal:** repetitive cough with whoops, vomiting; anoxia and severity of cough cause neurological damage and eye hemorrhages; organism present at beginning disappears
- (>3 weeks) **convalescence:** less cough, secondary complications manifest: pneumonia, seizures, encephalopathy

Treatment

Supportive care; hospitalization if <6 months old, erythromycin

Prevention

- Vaccine: DTaP; immunity wanes 5–7 years
- Babies born with little or no immunity (IgA) from mom

GENUS: *FRANCISELLA*

- **Gram-negative small rods**
- **Facultative intracellular pathogen**

Francisella tularensis

Distinguishing Characteristics

- Small Gram-negative rod
- Serodiagnosis; culture is hazardous

Reservoir

Many species of wild **animals**, especially rabbits, deer, and rodents

Transmission

- **Tick bite** (*Dermacentor*) → **ulceroglandular** disease, characterized by **fever**, ulcer at bite site, and regional lymph node enlargement and necrosis
- **Traumatic implantation** while skinning rabbits → ulceroglandular disease
- Aerosols (skinning rabbits) → pneumonia
- Ingestion (of undercooked, infected meat or contaminated water) produces typhoidal tularemia.

Pathogenesis

- **Facultative intracellular pathogen (localizes in reticuloendothelial cells)**
- Granulomatous response

Disease

Tularemia

- Endemic in every state of U.S.
- Arkansas and Missouri highest

Treatment

Streptomycin

Prevention

- Protect against tick bites, gloves while butchering rabbits
- Live, attenuated vaccine for persons in high-risk occupations

Note

Zoonotic organisms
- *Brucella*
- *Bacillus anthracis*
- *Listeria monocytogenes*
- *Salmonella enteritidis*
- *Campylobacter*
- Q fever *(Coxiella burnetii)*
- *Chlamydia psittaci*
- *Francisella tularensis*

GENUS: *BRUCELLA*

- **Gram-negative rods**
- **Zoonotic**
- **Facultative intracellular pathogen**

Brucella species

Distinguishing Characteristics

- Small **Gram-negative** rods, aerobic
- Facultative intracellular
- Serological confirmation of disease most common
- Culture is hazardous.

Reservoir

Domestic livestock

Transmission

- **Unpasteurized dairy products**
- **Direct contact with the animal**, work in slaughterhouse

Pathogenesis

- **Endotoxin**
- **Facultative intracellular parasite** (localizes in cells of reticuloendothelial system, RES) → **septicemia**
- **Granulomatous response** with central necrosis

Disease

Brucellosis (undulant fever)
- ***B. abortus*: cattle**
- ***B. suis*: pigs**
- ***B. melitensis*: goats**

Acute Septicemias
- Fever 100–104°F (often in evening)
- Influenza-like symptoms, including arthralgias, myalgia, back pain
- Sweating
- Hepatomegaly

Undulant Form: Milder, often a result of incomplete treatment

Chronic Form
(Disease for more than one year.)

- Usually in older people (veterinarians)
- Cyclic bouts of depression and sweating
- Fever rare
- Ocular complications (uveitis) in 5–10%

Prevention

- Vaccinate cattle; vaccinate high-risk humans.
- Pasteurize milk, especially goat milk.

GENUS: *CAMPYLOBACTER*

- **Gram-negative curved rod with polar flagella**
- **Microaerophilic**

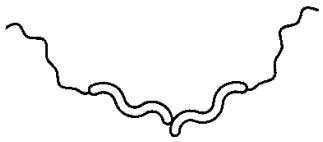


Figure I-2-10. Campylobacter

Campylobacter jejuni

Distinguishing Characteristics

- Motile, curved Gram-negative rods ("gulls' wings")
- Microaerophilic, grows well at 42°C on selective media (Campy medium or Skirrow's agar); oxidase positive.

Reservoir

Intestinal tracts of humans, cattle, sheep, dogs, cats, **poultry**

Transmission

Fecal-oral, primarily from **poultry**

Pathogenesis

- **Low infectious dose (as few as 500)**
- **Invades** mucosa of the colon, destroying mucosal surfaces; blood and **pus** in stools (inflammatory diarrhea)
- Rarely penetrates to cause septicemia

Disease

- Common cause of infectious diarrhea worldwide
- In U.S., *Campylobacter* enteritis > (*Salmonella* + *Shigella*)
- **Ten to more stools/day, may be frankly bloody**
- Abdominal pain, fever, malaise, nausea, and vomiting
- Generally **self-limiting in 3–5 days** but may last longer
- Complications:
  - **Guillain-Barré syndrome** (~30% of the GBS in the U.S.).
  - Reactive arthritis

Treatment

Erythromycin, fluoroquinolones, penicillin-resistant

Helicobacter pylori

Distinguishing Characteristics

- Gram-negative spiral gastric bacilli with flagella
- Microaerophilic, 37°C growth (Campy medium or Skirrow's agar); oxidase positive.

Reservoir

Humans

Transmission

Fecal-oral, oral-oral

Pathogenesis

- **Motile**
- **Urease-positive**: ammonium cloud neutralizes stomach acid, allowing survival in stomach acid during transit to border.
- **Mucinase** aids in penetration of mucous layer (rapid shift down to neutral as it penetrates).
- **Invasive** into stomach lining where pH is neutral.
- Inflammation is prominent.
- Two biotypes (I and II); type I produces vacuolating cytotoxin.

Disease

- Causes chronic **gastritis and duodenal ulcers.**
- Associated with several forms of **stomach cancer.** (Atrophic gastritis rather than duodenal ulcers correlates with risk.)
- Now classed by WHO as **Type I carcinogen.**

Lab Diagnosis

- Serologic test
- Biopsy with culture; histology with Giemsa or silver stain
- Breath test: ^{13}C-urea swallowed; ammonia + ^{13}C-CO_2 produced

Treatment

Myriad of regimens.)

Omeprazole + amoxicillin + clarithromycin

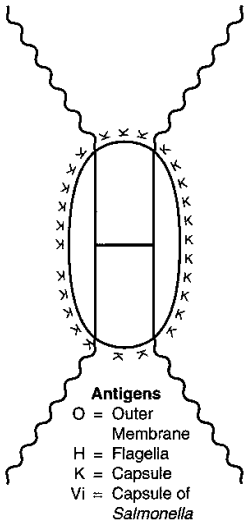


Figure I-2-11. Antigens of Enterobacteriacae

FAMILY: *ENTEROBACTERIACEAE*

- **Gram-negative rods**
- **Facultative anaerobes**
- Ferment glucose
- **Cytochrome C oxidase negative**
- **Reduce nitrates to nitrites**
- Catalase positive

Pathogenesis (overall for family)

- **Endotoxin**
- Some also produce **exotoxins.**
- **Antigens**
  - O = cell envelope or O antigen
  - H = flagellar (motile cells only) antigen
  - K = capsular polysaccharide antigen
  - Vi = *Salmonella* capsular antigen (virulence)

Lab Diagnosis

Grow on two media:

- Blood
- Eosin-methylene blue or MacConkey agar (differentiate lactose fermentation)

Genera in Family

- **Lactose fermenters: CEEK ("colored" colonies)**

  Citrobacter

  Escherichia

  Enterobacter

  Klebsiella

- **Nonlactose fermenters: ShYPS ("colorless" colonies [same color as original media])**

  Shigella, *Yersinia* } nonmotile, non H_2S producers

  Proteus, *Salmonella* } motile, H_2S producers

Slow lactose fermenter: *Serratia,* which produces a salmon red pigment and is an opportunist.

Each genus is characterized by a whole series of chemical tests to detect the presence of certain enzymes or pathways.

GENUS: *ESCHERICHIA*

- **Gram-negative rod**
- ***Enterobacteriaceae***
- **Ferments lactose**

Escherichia coli

Distinguishing Characteristics

- **Gram-negative rod**
- **Facultative anaerobic, oxidase negative**
- *E. coli* is **lactose-fermenter**: colonies with iridescent green sheen on EMB

Reservoirs

- **Human colon**; may colonize vagina or urethra
- Contaminate crops where human fecal fertilizer is used
- **Enterohemorrhagic strains: bovine feces**

Diseases

Urinary Tract Infections (UTIs)

- *E. coli* is most common cause.
- Transmission: from own fecal flora → urethra

  Predisposing factors: female anatomy; strictures, stones, or abnormality in urine flow; indwelling urinary catheters
- Pathogenesis
  - Motility aid
  - Adherence to uroepithelium important: **P-pili: pyelonephritis-associated pili** (PAP or P-pili); X-adhesins
  - β-hemolytic (many)
- Treatment: ampicillin or sulfonamides for UTI

Neonatal Septicemia and Meningitis

- Second most common cause after Group B streptococci
- Maternal fecal flora → vagina contaminates baby
  - **Capsule:** most strains have K1 serotype. Capsule allows the organism to evade phagocytic uptake in the blood and spleen so organism can get to blood/brain barrier.
  - **Endotoxin:** triggers **shock**; **inflammation** at blood/brain barrier **facilitates invasion.**

Septicemia

- Indwelling IV lines predispose to invasion from skin.
- Cytotoxic drugs damage intestinal mucosa, allowing escape.
- **Endotoxin** triggers Gram-negative shock.

Note

***E. coli* Identification from Stool**

- Isolation of *E. coli* from feces is not by itself significant.
- Sorbitol MacConkey screen.
- Most *E. coli* ferment sorbitol.
- Most EHEC do not (colorless).

Animal models and tissue culture assays may be used, but other methods of differentiating pathogenic *E. coli* from normal flora are more commonly:

- Immunoassay looking for specific protein antigens (on or excreted by the bacterium)
- Serotyping since certain serotypes are more often pathogenic
- DNA probes for specific genes in a culture
- PCR on clinical specimen

Note

Mnemonic:

Toxins ↑cAMP

c = cholera
A = anthrax
Σ = *E. coli* LT
P = pertussis

Gastroenteritides/Diarrheas

- **Most strains:** fecal-oral spread; poor sanitation/water; use of human feces on vegetables or fruits.
- **Enterohemorrhagic strains**: bovine fecal contamination: undercooked hamburger, raw milk, apple juice (from fallen apples), alfalfa sprouts.

ETEC = Enterotoxigenic *E. coli*

Major cause of "Traveler's Diarrhea" and diarrhea in <3 year olds in developing countries.

- Two enterotoxins are produced:

  LT: The **heat labile toxin** stimulates adenylate cyclase by **ADP ribosylation of G_s** resulting in increased cAMP causes outflow of chloride ions and water in small intestine → watery diarrhea.

  ST: The **heat stabile toxin** causes diarrhea by stimulating guanylate cyclase.
- Enterotoxin production identified by immunoassay, bioassay, or DNA probe assays.
- **Capsule** impedes phagocytosis
- **Colonizing factor adhesins** (CFAs): bind to small intestine.

EPEC = Enteropathogenic *E. coli*

- Second most common cause of infantile diarrhea after rotavirus
- **Prolonged, watery diarrhea** in babies <1 year in developing countries
- Plasmid containing virulence genes called the *E. coli* adherence factor (EAF); EPEC adhere to M cells, causing rearrangement of actin and effacement of the brush border microvilli.

EIEC = Enteroinvasive *E. coli*

- **Invades** large bowel, similar to shigellosis, including the formation of actin "jet trails"
- Often manifests as **watery diarrhea** (with **excess of leukocytes**, however) with **fever** and **abdominal** pain. Only about 10% progress on to dysentery.

EHEC = Enterohemorrhagic *E. coli*

- Also known as **verotoxin producing** *E. coli* (VTEC)
- **O157:H7** is the most common serotype.
- **EHEC** disease ranges from **mild diarrhea** without blood **to hemorrhagic colitis ± hemolytic uremic syndrome** (HUS). **Fever is generally absent,** distinguishing it from shigellosis. HUS most common in kids <5 years old.
- Plasmid-associated **verotoxins = Shigalike toxin 1 and 2. Both toxins inhibit protein synthesis in the large intestine by nicking the 60S ribosome.**
- Not invasive, no inflammatory response; afebrile
- Focus of infection is the large intestine.
- Excess fecal leukocytes are not seen. Damage is due to toxin activity so does not promote inflammatory response.

  Lab screen: most **EHEC** are sorbitol non-fermenters so have colorless colonies on sorbitol MacConkey; DNA probe for verotoxin genes.
- Antibiotics may increase risk of HUS and kidney damage, especially early and in children. Check before using.

EaggEC = Enteroaggregative *E. coli*

- Important cause of **persistent diarrhea** with vomiting, low-grade fever in the developing world.
- **Fimbriae** create a "stacked brick-like" biofilm by attaching to each other and enterocytes.
- Also produce enterotoxin (EAST1).

Treatment

- Depends on strain, severity of diarrhea, presence of fever, blood, and pus
- Rehydration for traveler's diarrhea; trimethoprim-sulfamethoxazole may shorten duration of symptoms
- No antibiotic currently recommended for 0157:H7 (generally bloody diarrhea without fever)
- Fluoroquinolones for bloody diarrhea with pus and fever >101°F and tenesmus
- Antibiotic resistance primarily mediated by plasmid-encoded enzymes, e.g., β-lactamase, and aminoglycoside modifying enzymes

GENUS: *SHIGELLA*

- **Gram-negative rod**
- ***Enterobacteriaceae***
- **Nonlactose fermenter**
- **Nonmotile**

Shigella species

S. dysenteriae (most severe disease), *S. sonnei* (most common in U.S.), etc.

Distinguishing Characteristics

- **Gram-negative rods**, non-motile
- Facultative anaerobes, **non-lactose fermenting** (colorless colonies on EMB or MacConkey)
- Identified by biochemical reactions or by serology with anti-O antibody in agglutination test.

Reservoir

Human colon only (no animal carriers)

Transmission

- **Fecal-oral spread**, person to person
- As with all fecal-oral spread, the "Four Fs": fingers, food, feces, flies

Pathogenesis

- **Endotoxin** triggers inflammation.
- No H antigens
- Shigellae **invade M cells** (membrane ruffling and macropinocytosis); get into the cytoplasm, replicate and then **polymerize actin jet trails to go laterally** without going back out into the extracellular milieu. This produces **very shallow ulcers** and rarely causes invasion of blood vessels.
- **Shiga toxin:**
  - Produced by *S. dysenteriae*, type 1
  - Three activities: **neurotoxic, cytotoxic, enterotoxic**
  - **AB component toxin** is internalized in human cells; **inhibits protein synthesis by clipping 60 ribosomal subunit.**

Disease

Enterocolitis/Shigellosis (most severe form is dysentery)

- Few organisms required to start infection (1–10) (extremely acid resistant)
- 1–4 day incubation
- Organisms invade producing bloody diarrhea.
- **Fever (generally >101°F), lower abdominal cramps, tenesmus; diarrhea first watery, then bloody, invasive but rarely septicemia; shallow ulcers.**
- Severity depends on the age of patient and the strain; *S. dysenteriae* Type 1 with toxin most severe.

Treatment

- Mild cases: fluid and electrolyte replacement only
- Severe cases: antibiotics
- Resistance is mediated by plasmid-encoded enzymes

Prevention

- Proper sanitation (sewage, clean drinking water, hand washing)

Note

Comparative Microbiology

- Invasive bacteria: PMN in stool: *Shigella, Salmonella, Campylobacter*, EIEC.
- Toxigenic bacteria: ETEC, *V. cholera, Cl. perfringens*, EHEC.

GENUS: *KLEBSIELLA*

- **Gram-negative rod**
- ***Enterobacteriaceae***
- **Major capsule**

Klebsiella pneumoniae

Distinguishing Characteristics

- **Gram-negative rods** with **large** polysaccharide **capsule**
- **Mucoid, lactose-fermenting** colonies on MacConkey agar
- Oxidase negative

Reservoir

Human colon and upper respiratory tract

Transmission

All commonly from own flora

Pathogenesis

- **Capsule:** impedes phagocytosis
- **Endotoxin:** causes fever, inflammation, and shock (septicemia)

Transmission/Disease

Pneumonia

- Community-acquired, most often in older males; most commonly in **patients with either chronic lung disease or alcoholism, or diabetes** (but not the most common cause of pneumonia in alcoholics! *S. pneumoniae* is.)
- Endogenous; assumed to reach lungs by inhalation of respiratory droplets from upper respiratory tract.
- Frequent **abscesses** make it hard to treat; fatality rate high
- **Sputum is generally thick and bloody (currant jelly)** but not foul smelling as in anaerobic aspiration pneumonia.

Urinary Tract Infections: Catheter-related (nosocomial) from fecal contamination of catheters

Septicemia: In immunocompromised patients may originate from bowel defects or invasion of IV lines

Treatment

Antibiotic sensitivity testing must be done

Prevention

Good catheter care; limit catheter use

In A Nutshell

Comparative Microbiology: Major encapsulated organisms

Some Killers Have Pretty Nice Capsules:

Strep pneumoniae

Klebsiella pneumoniae

Haemophilus influenzae Type b (a-d)

Pseudomonas aeruginosa

Neisseria meningitidis

Cryptococcus neoformans (the yeast)

(Not a complete list, just the big ones!)

GENUS: *SALMONELLA*

- **Gram-negative rod (*Enterobacteriaceae*)**
- **Non-lactose fermenter**
- **Motile**

2,000 serotypes of salmonellae; serotype names are still in use.

Diseases

Enteric or Typhoid Fever (*S. typhi*)
Gastroenteritis
Septicemia

Salmonella typhi

Distinguishing Characteristics

- **Gram-negative rods**, highly motile with the **Vi capsule**
- Facultative anaerobe, **non-lactose fermenting**
- **Produces H_2S**
- Species identification with biochemical reactions
- **Sensitive to acid**

Reservoir

- **Humans only; NO ANIMAL RESERVOIRS**
- Only the typhoid Mary's of the world!

Transmission

- **Fecal-oral** route from **human carriers (gall bladder)**
- **Decreased stomach acid or** impairment of mononuclear cells such as in **sickle cell disease predisposes** to *Salmonella* infections

Pathogenesis/Disease

Typhoid Fever (Enteric Fever), *S. typhi*
(milder form: paratyphoid fever; *S. paratyphi*)

- Organism ingested (large number if stomach acid is normal).
- **Infection begins in ileocecal region;** constipation common.
- Host cell membranes "ruffle" from *Salmonella* contact.
- *Salmonella* reach basolateral side of M cells then **mesenteric lymph nodes and blood** (transient 1' septicemia)
- At 1 week: patients have 80% **positive blood cultures;** 25% have **rose spots** (trunk/abdomen)
- **Liver and spleen** are infected with additional release of bacteria to bloodstream → signs of **septicemia** (mainly fever)

- *S. typhi* survives intracellularly and replicates in macrophages; **resistant to macrophage killing due to:**
  - Decreased fusion of lysosomes with phagosomes
  - Defensins (proteins) allow it to withstand oxygen-dependent and -independent killing.
- Released from the macrophages. The Vi capsular antigen (*S. typhi* only) withstands complement-mediated killing.
- **Biliary system** (liver, gall bladder) is infected, organisms enter intestinal tract in bile.
- **By week 3: 85% of stool cultures** are positive.
- Symptoms: **fever,** headache, abdominal pain, constipation more common than diarrhea
- Complications of untreated: **necrosis of Peyer's patches** with perforation (local endotoxin triggered damage), thrombophlebitis, cholecystitis, pneumonia, abscess formation, etc.

Treatment

Ciprofloxacin or ceftriaxone in seriously ill; some drug resistance

Prevention

Sanitation; 3 vaccines: attenuated oral vaccine of *S. typhi* strain 21 (Ty21a), parenteral heat killed *S. typhi,* and parenteral Vi polysaccharide capsular vaccine

Nontyphoidal Salmonellae: *S. enteritidis, S. typhimurium,* and Other Species

Distinguishing Characteristics

- Facultative Gram-negative rods, non-lactose-fermenting on EMB, MacConkey medium
- Produces H_2S, **motile** (unlike *Shigella*)
- Speciated with biochemical reactions and serotyped with O, H, and Vi antigens
- **Antibodies** to O, Vi, and H antigens **in patient's serum** can be detected by agglutination (**Widal test**).

Reservoir

Enteric tracts of human and domestic animals, e.g., **chickens** and turtles

Transmission

Largely through chicken products (raw chicken and eggs) in the kitchen

Pathogenesis

- Sensitive to stomach acid (infectious dose 10^5 organisms)
- Lowered stomach acidity (antacids or gastrectomy) increases risk.
- Endotoxin in cell wall; no exotoxin
- **Invades** the mucosa in the ileocecal region, invasive to lamina propria → inflammation → increased PG → increased cAMP → loose diarrhea; shallow ulceration.
- Spread to septicemia not common with *S. enteritidis* (the most common) but may occur with others.

Diseases

Enterocolitis/Gastroenteritis
Second most common after *Campylobacter*: **6–48 hour incubation; nausea, vomiting, only occasionally bloody, loose stools, fever, abdominal pain, myalgia, headache**

Septicemia

- *S. cholerae-suis*, *S. paratyphi*, and *S. dublin*
- When it occurs, it is usually in very young or elderly.
- Endocarditis or arthritis complicate about 10%.

Osteomyelitis: Sickle cell disease predisposes to osteomyelitis. ***Salmonella*** is the **most common causative agent of osteomyelitis in sickle cell disease** (not trait) **patients (>80%).**

Treatment

- Antibiotics **generally not effective so not recommended** for uncomplicated enterocolitis.
- Antibiotics for septicemia depend on sensitivity tests.

Prevention

Proper sanitation (sewage, clean drinking water, hand washing, particularly food handlers)

GENUS: *YERSINIA*

- **Gram-negative rod**
- ***Enterobacteriaceae*** (oxidase negative)

Yersinia pestis (*Enterobacteriaceae*)

Distinguishing Characteristics

- Small **Gram-negative** rods with **bipolar staining**
- **Facultative intracellular parasite**
- **Coagulase +**
- Clinical specimens and **cultures are hazardous**
- **Serodiagnosis** or direct **immunofluorescence**

Reservoir

U.S.: desert southwest, rodents, e.g., prairie dogs, chipmunks, squirrels, field mice, and voles

Transmission

- Wild rodents **flea bite** → sylvatic plague
- Human-to-human transmission by **respiratory droplets**

Pathogenesis

- **Coagulase**-contaminated mouth parts of flea
- **Endotoxin** and an exotoxin
- **Two antigens (V and W)**
- **Envelope antigen (F-1) inhibits phagocytosis**

Disease

Bubonic Plague

- Flea bites infected animal and then later uninfected human: coagulase role-contaminated mouth parts
- Symptoms:
  - **Rapidly increasing fever**
  - **Regional buboes**
  - **Conjunctivitis**
  - Leads to septicemia and death if untreated

Pneumonic Plague

- Arises from septic pulmonary emboli in bubonic plague or **inhalation of organisms from infected individual.**
- **Highly contagious!**

Treatment

- Streptomycin with tetracycline
- **Strict quarantine for 72 hours** after starting antibiotics

Prevention

- Animal control; avoid sick and dead animals.
- **Killed vaccine** is available for high-risk occupations.

Yersinia enterocolitica

- **Zoonotic, unpasteurized milk, pork**
- Enterotoxin
- **Multiplies in the cold**
- **Enterocolitis** in northern climates (Michigan, Scandinavia)
- Presentations may vary with age
  - Very young: febrile diarrhea (blood and pus)
  - Older kids/young adults: **pseudoappendicitis**
  - Adults: enterocolitis with post-infective sequelae like reactive arthritis
- **Blood transfusion**-associated infections

GENUS: *PROTEUS*

- **Gram-negative rod**
- ***Enterobacteriaceae***
- **Peritrichous flagella**
- **Non-lactose-fermenting**
- **Urease positive**

Proteus mirabilis, Proteus vulgaris

Distinguishing Characteristics

- Gram-negative rods
- **Highly motile**; "swarming" motility on surface of blood agar
- **Urease** produced
- Facultative anaerobe (*Enterobacteriaceae*), oxidase negative

Reservoir

Human colon and environment (water and soil)

Disease

Urinary tract infection and septicemia

Note

Weil-Felix test: antigens of OX strains of *Proteus vulgaris* cross-react with rickettsial organisms.

Pathogenesis

- **Urease raises urine pH to cause kidney stones** (staghorn renal calculi)
- **Motility** may aid entry into bladder.
- Endotoxin causes fever and shock when septicemia occurs.

Treatment

Do susceptibilities.

Prevention

Promptly remove urinary tract catheters.

GENUS: *VIBRIO*

- **Gram-negative curved rod with polar flagella**
- Oxidase positive (*Vibrionaceae*)

Vibrio cholerae

- *Vibrio cholerae* O1 divided into biotype El Tor (predominant now) and Cholerae (classical)
- *Vibrio cholerae* O139 also produces cholera toxin.

Distinguishing Characteristics

- "Shooting star" motility inactivated by specific serum.
- **Oxidase-positive**, which distinguishes them from *Enterobacteriaceae*
- Growth on **alkaline** but not acidic media
  (**TCBS** = Thiosulfate Citrate Bile salt Sucrose medium)

Reservoir

Human colon; **no vertebrate animal carriers.** (Copepod or shellfish may be contaminated by water contamination.) Human carriage may persist after untreated infection for months after infection; permanent carrier state rare.

Transmission

- Fecal-oral spread; sensitive to stomach acid
- Requires high dose ($>10^7$ organisms), if stomach acid is normal.

Pathogenesis

- Motility, mucinase, and toxin co-regulated pili (Tcp) aid in attachment to the intestinal mucosa.
- **Cholera enterotoxin (choleragen) similar to *E. coli* LT. ADP ribosylates (G_s alpha) activating adenylate cyclase → increased cAMP → efflux of Cl^- & H_2O (persistent activation of adenyl cyclase).**

Disease

Cholera: Rice water stools, **tremendous fluid loss**; hypovolemic shock if not treated.

Treatment

- **Fluid and electrolyte replacement**
- Doxycycline or ciprofloxacin shortens disease and reduces carriage.

Prevention

Proper sanitation; new vaccine; tetracycline to reduce transmission

Vibrio parahaemolyticus

- Food poisoning associated with **undercooked or raw seafood**
- **Marine**
- 5–94 hours incubation (mean 24 hours)
- Self-limiting watery diarrhea with cramping, abdominal pain

Vibrio vulnificus

- Brackish water; **oysters**; warm months
- **Cellulitis** when it gets into cuts; hard to treat
- **Gastroenteritis** when ingested
- **Septicemia** in patients with preexisting liver disease (50% fatal)

GENUS: *PASTEURELLA*

- **Small Gram-negative rods**
- **Facultative anaerobic rods**

Pasteurella multocida

Distinguishing Characteristics

Small **Gram-negative** rods

Reservoir

Mouths of many animals, especially cats and dogs

Transmission

Animal bites; particularly from cat bites

Disease

Wound infection leading to **cellulitis with lymphadenitis**

Pathogenesis

Endotoxin, capsule; spreads rapidly within skin, no exotoxins known

Lab Diagnosis

Rarely cultured because routine prophylaxis is common.

Treatment/Prevention

Amoxicillin/clavulanate for cat bites. Amoxicillin/Clavulanate is standard prophylaxis and treatment for most bites (human included), along with thorough cleaning.

GENUS: *HAEMOPHILUS*

- **Gram-negative, pleomorphic rod**
- **Requires growth factors**

Haemophilus influenzae

Distinguishing Characteristics

- Encapsulated, **Gram-negative rod**
- Fastidious: requires **factors X (hemin)** and **V (NAD)** for growth on nutrient or blood agar
- Grows near ***S. aureus* on BA = "Satellite" phenomenon**
- Chocolate agar provides both X and V factors

Reservoir

Human nasopharynx

Transmission

Respiratory droplets, shared toys

Pathogenesis

- **Polysaccharide capsule (type b capsule is polyribitol phosphate)** most important virulence factor
- 95% of invasive disease is caused by capsular type b. Capsule important in diagnosis.
  Antigen screen on CSF (e.g., latex particle agglutination); serotype all isolates by Quellung.
- **IgA protease** is a mucosal colonizing factor.

Diseases

Meningitis: *H. influenzae*; type b encapsulated strains.

Epidemic in unvaccinated children ages 3 months to 2 years

- After maternal antibody has waned
- Before the immune response of the child is adequate
- *H. influenzae* **was** most common cause of meningitis in 1 to 5-year-old children (mainly younger than 2) up to 1990.
- Still a problem if child is <2 years and not vaccinated.

Vaccination effective to prevent type b disease.

- **Polyribitol capsule conjugated to protein:**
  (diphtheria toxoid or *N. meningitidis* outer membrane proteins)
  making it a **T cell dependent vaccine.**
- Vaccine: 2, 4, 6 months; booster 15 months; 95% effective

Otitis Media
Usually nontypeable strains

Bronchitis
Exacerbations of acute bronchitis in smokers with COPD

Pneumonia
1–24 months; smokers

Epiglottitis
Rare in vaccinated kids; seen in unvaccinated toddlers. ***H. influenzae*** **was the major causative agent.**

Treatment

Cefotaxime or ceftriaxone for empirical therapy of meningitis. Check nasal carriage before releasing; use rifampin if still colonized.

Prevention

- **Conjugate capsular polysaccharide-protein vaccine**
- Rifampin reduces oropharynx colonization and prevents meningitis in unvaccinated, close contacts <2 years.

Haemophilus ducreyi

- Sexually transmitted disease.
- **Chancroid** (genital ulcers): **soft, painful chancre**
  ("You do cry with ducreyi.")
- **Slow to heal without treatment**
- **Open lesions increase transmission of HIV**
- Diagnosis: **DNA probe**

GENUS: *BACTEROIDES/PREVOTELLA*

- **Gram-negative rod**
- **Anaerobic**
- Modified LPS with reduced activity

Bacteroides fragilis

Distinguishing Characteristics

- **Anaerobic**, Gram-negative rods
- Anaerobes are identified by biochemical tests and gas chromatography.

Reservoir

Human **colon; the genus *Bacteroides* is the predominant anaerobe.**

Transmission

Endogenous from bowel defects (e.g., from cytotoxic drug use, cancer), surgery, or trauma

Pathogenesis

- Modified LPS (missing heptose and 2-keto-3 deoxyoctonate) has reduced endotoxin activity.
- Capsule is antiphagocytic.

Disease

Septicemia, peritonitis (often mixed infections), and abdominal abscess

Treatment

- Metronidazole, clindamycin, or cefoxitin. **Abscesses should be surgically drained.**
- **Antibiotic resistance** is common (penicillin G, some cephalosporins, and aminoglycosides).

Prevention

Prophylactic antibiotics for gastrointestinal or biliary tract surgery

Bacteroides melaninogenicus = Prevotella melaninogenica

- **Melanin-producing (black) *Bacteroides***
- Normal gingival flora
- Anaerobe
- Oral abscesses
- Heparinase leads to clotting in brain.

GENUS: *TREPONEMA*

- **Spirochetes: spiral with axial filament (endoflagellum)**
- **Poorly visible on Gram stain but basically Gram-negative**

Treponema pallidum

Distinguishing Characteristics

- **Thin spirochete**, not reliably seen on Gram stain
- Basically a Gram-negative cell envelope
- Outer membrane has endotoxin-like lipids.
- Axial filaments = endoflagella = periplasmic flagella
- Cannot culture in clinical lab; serodiagnosis
- Is an **obligate pathogen (but not intracellular)**

Reservoir/Transmission

Human genital tract; transmitted **sexually** or **across the placenta.**

Pathogenesis

Disease characterized by **endarteritis resulting in lesions.** Strong tendency to chronicity.

Disease

Syphilis: Progression in untreated syphilis: **incubation**: 10–90 days.

Primary

- Nontender chancre(s) at site of inoculation
- Margins generally "clean," distinctly indurated edge
- **Contagious (but you still cannot culture!)**
- Chancre is good source of material for microscopy. (This is important as only 50% of those with chancres will be positive by nontreponemal serologic tests.)
- Heals spontaneously in 3–6 weeks

Secondary

- 1 to 3 months later (*T. pallidum* has spread early via bloodstream.)
- **Maculopapular** (often copper colored) **rash on skin including palms and soles** ± patchy alopecia
- Flat wart-like perianal **condylomata lata** and **mucous membrane lesions, both highly infectious**
- May "heal" spontaneously, regress (several times), and finally heal.
- Serology is almost always strongly reactive.

Latent syphilis

- Positive serology only

Tertiary

- May be years later in about one-third of the untreated patients
- Tertiary lesions consist of gummas, aortitis, or central nervous system inflammation.
- VDRL may be negative.

Congenital syphilis

- Commonly in babies of IV drug abusing women
- Sx: stillbirths, multiple fetal abnormalities (keratitis, 8th nerve deafness, notched teeth), or sometimes asymptomatic (or snuffles) at birth until two.

Laboratory Diagnosis

Visualize organisms by immunofluorescence or dark-field microscopy.

Serology important: Two types of antibody:

1. **Nontreponemal antibody (= reagin)**
   - **Binds to cardiolipin**
     - An antigen found in mammalian mitochondrial membranes and in treponemes (but probably are from the host since treponemes don't make).
     - Cheap source of antigen is cow heart, which is used in screening tests (VDRL, RPR, ART).
   - **Screening tests: Nontreponemal antibody tests:**
     - Venereal Disease Research Lab (VDRL)
     - Rapid plasma reagin (RPR)
     - Automated Reagin Test (ART)
     - Any one may be used for screening (inexpensive).
     - Very sensitive in primary (except early) and secondary; titer may decline in tertiary and with treatment.
     - But not specific; confirm with FTA-ABS
2. **Treponemal antibody**

   Earliest antibody; **binds to spirochetes**

   Specific tests for *Treponema* (more expensive)
   - Fluorescent Treponemal Antibody-Absorption (**FTA-ABS**; most widely used test)
   - *Treponema pallidum* Microhemagglutination
   - These tests are more specific and positive earlier; usually remains positive for life. But positive in patients with other treponeme disease (bejel) and may be positive in Lyme disease.

TORCH screen for neonates no longer includes syphilis test; must order separately.

Treatment

Benzathine penicillin (long-acting form) for primary and secondary syphilis (no resistance to penicillin)

Prevention

Benzathine penicillin is given to contacts, no vaccine is available.

Jarisch-Herxheimer Reaction

- Starts generally during the first 24 hours of antibiotic treatment
- Increase in temperature, decrease in blood pressure; rigors, leukopenia
- May occur during treatment of any of the spirochete diseases

GENUS: *BORRELIA*

- **Larger spirochetes**
- **Gram-negative**
- **Microaerophilic**

Borrelia burgdorferi

Distinguishing Characteristics

Spirochete, not seen well on Gram-stained smear; can be cultured

Reservoir

Two reservoirs: **white-footed mice (nymphs)** and **white-tailed deer (adult ticks)**

Transmission

By *Ixodes* (deer) ticks and nymphs; worldwide but in three main areas in the U.S.:

- ***Ixodes scapularis*** (*I. dammini*) in Northeast (e.g., Connecticut), Midwest (e.g., Wisconsin)
- ***Ixodes pacificus*** on West Coast (e.g., California)

Pathogenesis

- *B. burgdorferi* **invades skin and spreads via the bloodstream to involve primarily the heart, joints, and central nervous system.**
- Arthritis is caused by immune complexes.

Disease

Lyme Disease

Initial symptoms:

- **Erythema (chronicum) migrans: spreading annular skin lesion with an erythematous leading edge and central clearing**, "bull's eye" seen in 85% of cases.
- Malaise, headache, severe fatigue, fever, chills
- Musculoskeletal pain, lymphadenopathy

One to several weeks dissemination:

- **Neurologic**: severe headache, meningitis, cranial nerve palsies (**Bell's palsy**)
- **Cardiac**: arrhythmias, myocarditis, pericarditis

Late and lasting for months or years:

- **Arthralgias, arthritis** (sx in 80% within few weeks to 2 years)

Laboratory Diagnosis

- **Serodiagnosis** by detecting IgM or IgG antibody (many false negatives/some false positives)
- **Amplification/probes and cultures** (Kelly medium) available

Treatment

- Doxycycline, amoxicillin, or azithromycin/clarithromycin (primary)
- Ceftriaxone for secondary
- Doxycycline or ceftriaxone for arthritis

Prevention

- DEET; avoid tick bite
- New vaccine: ospA flagellar antigen

GENUS: *LEPTOSPIRA*

- **Spirochetes: thin, with hooks**
- **Too thin to visualize, but Gram-negative cell envelope**

Leptospira interrogans

Distinguishing Characteristics

- **Spirochetes with tight terminal hooks**
  - seen on **dark-field microscopy** but not light microscopy
  - can be cultured *in vitro*; aerobic
- Generally diagnosed by serology

Reservoir

Wild and domestic animals

Transmission

- **Via animal urine in water**
- In U.S., via **dog, livestock, and rat urine** through contaminated **recreational waters (jet skiers) or occupational exposure (sewer workers)**

Pathogenesis

No toxins or virulence factors known.

Disease

Leptospirosis

- **Influenza-like disease ± GI tract symptoms**
- Progressing on to hepatitis and renal failure if not treated

Laboratory Diagnosis

Serodiagnosis and dark-field microscopy

Treatment

Penicillin G or doxycycline

Prevention

- Doxycycline effective for short-term exposure
- Vaccination of domestic livestock and pets; rat control

Table I-2-24. Comparison of the Genera *Rickettsia, Chlamydia,* and *Mycoplasma* with Typical Bacteria

	Typical Bacteria (*S. aureus*)	*Chlamydia*	*Rickettsia*	*Mycoplasma*
Obligate intracellular parasite?	Most no	Yes	Yes	No
Make ATP?	Normal ATP	No ATP	Limited ATP	Normal ATP
Peptidoglycan layer in cell envelope?	Normal peptidoglycan	Modified* peptidoglycan	Normal peptidoglycan	No peptidoglycan

*Chlamydial peptidoglycan lacks muramic acid and is considered by some as modified, by others as absent.

Table I-2-25. Infections Caused by Rickettsiae and Close Relatives

Group Disease	Bacterium	Arthropod Vector	Reservoir Host
Spotted Fevers:			
Rocky Mountain Spotted Fever	***Rickettsia rickettsii***	**Ticks**	**Dogs, rodents, ticks**
Rickettsialpox	*Rickettsia akari*	Mites	Mice
Typhus Group:			
Epidemic	*Rickettsia prowazekii*	Human louse	Humans
Endemic	*Rickettsia typhi*	Fleas	Rodents
Scrub	*Rickettsia tsutsugamushi*	Mites	Rodents
Others:			
Q fever	***Coxiella burnetii***	**None**	**Cattle, sheep, goats**
Bacillary angiomatosis in AIDS	*Bartonella quintana* and *B. henselae (Rochalimaea)*	Human louse	Humans
Cat Scratch Fever	***Bartonella henselae (Rochalimaea)***	**None**	**Cats**
Human granulocytic or monocytic Ehrlichiosis*	*Ehrlichia*	*Ixodes* ticks + ?	?

* New tick-borne rickettsial disease caused by *Ehrlichia.*
Note: Co-infections from a single tick infected with more than one agent may occur: N. East U.S.A.: *Babesia* and *B. burgdorferi.* N. Central U.S.A.: *B. burgdorferi* and *Ehrlichia.*

GENUS: *RICKETTSIA*

Obligate intracellular bacteria

Rickettsia rickettsii

Distinguishing Characteristics

- **Obligate intracellular** bacteria that divide by binary fission and cannot make sufficient ATP.
- Not seen well on Gram-stained smear (too small), but have **Gram-negative cell envelope.**
- Cross-reaction of *Rickettsia* antigens with OX strains of *P. vulgaris* (Weil-Felix reaction)

Reservoir

Small wild rodents and larger wild and domestic animals (dogs)

Transmission

Hard ticks: *Dermacentor* (also reservoir hosts because of transovarian transmission)

Pathogenesis

Rickettsia **invades endothelial lining of capillaries, causing vasculitis.**

Disease

Rocky Mountain Spotted Fever

- Prevalent on **East Coast** (2–12 day incubation)
- Headache, fever, malaise, myalgias, toxicity, vomiting, and confusion
- **Rash** (maculopapular → **petechial**) **starts** (by day 6 of illness) **on ankles and wrists** and then spreads to the trunk, **palms, soles, and face** (**centripetal rash**).
- **Ankle and wrist swelling** also occur.
- Dx may be confused by gastrointestinal symptoms, periorbital swelling, stiff neck, conjunctivitis and arthralgias.

Diagnosis

- Clinical symptoms (above) and tick bite
- **Start treatment without laboratory confirmation.**
- Serodiagnosis by complement fixation or Weil-Felix test

Treatment

Doxycycline

Prevention

Tick protection and prompt removal; doxycycline effective in exposed persons

GENUS: *COXIELLA*

- **Obligate intracellular bacteria**
- **Two antigenic phases, one resistant to drying**

Coxiella burnetii

Distinguishing Characteristics

- Obligate intracellular bacterium
- Not seen well on gram-stained smear

Reservoir

Domestic livestock: **pregnant animals have high titers.**

Transmission

- **Inhalation of aerosols of urine, feces, amniotic fluid, or placental tissue**
- **Survives drying:** can be infective miles away
- No significant arthropod vector in human infection

Disease

Q fever

- **Febrile illness with NO RASH**
- **Pneumonia with hepatitis**

Pathogenesis

Intracellular

Lab Diagnosis

Serodiagnosis; **Weil-Felix test is negative.**

Treatment

Doxycycline or erythromycin

Prevention

Vaccine for high-risk occupations

GENUS: *EHRLICHIA*

- **Obligate intracellular bacteria**
- **Rickettsial family**

Ehrlichia chaffeensis

- Reservoir: ticks and deer
- Transmitted by the Lone Star tick (*Amblyomma)*
- Infects monocytes and macrophages
- **Human monocytic ehrlichiosis**

***Ehrlichia* (near relative of *E. equi*)**

- Reservoir: ticks, deer, mice
- Transmitted by the *Ixodes* tick
- Infects primarily neutrophils
- **Human granulocytic ehrlichiosis**

Disease

Ehrlichiosis

- **Similar to Rocky Mountain spotted fever** but generally without rash
- Leukopenia or low platelets
- Morulae (mulberry-like structures inside infected cells)

GENUS: *CHLAMYDIA*

- **Obligate intracellular bacteria**
- **Elementary body/reticulate body**
- **Not seen on Gram stain**
- **Cannot make ATP**
- **Cell wall lacks muramic acid**

Chlamydia trachomatis

Distinguishing Characteristics

- Obligate intracellular bacterium; cannot make ATP.
- Found **in cells as metabolically active, replicating reticulate bodies.**
- **Infective form:** inactive, extracellular **elementary body**
- Not seen on Gram stain; peptidoglycan layer lacks muramic acid.

Reservoir

Human genital tract and eyes

Transmission

Sexual contact and **at birth.** Trachoma is transmitted by **hand-to-eye contact.** Flies (trachoma).

Pathogenesis

Infection of nonciliated columnar or cuboidal epithelial cells of mucosal surfaces leads to **granulomatous response and damage.**

Diseases

STDs in U.S.

- **Serotypes D-K** (This is the most common bacterial STD in U.S. Herpes and HPV are more common.)
- **Nongonococcal urethritis, cervicitis, PID,** and major portion of infertility (no resistance to reinfection)
- **Inclusion conjunctivitis**
- **Inclusion conjunctivitis** and/or **pneumonia in neonates/infants** (**staccato cough**)

Lymphogranuloma venereum

- Serotypes L1, 2, 3
  - STD is prevalent in Africa, Asia, and South America
  - Swollen lymph nodes leading to genital elephantiasis in late stages
  - Tertiary: ulcers, fistulas, genital elephantiasis.

Trachoma

- Leading cause of preventable infectious blindness, **Serotypes A, B, Ba, and C**
- **Follicular conjunctivitis** leading to **conjunctival scarring** and **inturned eyelashes** leading to **corneal scarring** and **blindness.**

Lab Diagnosis

- DNA probes in U.S.
- **Cytoplasmic inclusions seen on Giemsa-, iodine-, or fluorescent-antibody-stained smear or scrapings**
- **Cannot be cultured on inert media**
- Is cultured in **tissue cultures or embryonated eggs**
- Serodiagnosis: complement fixation or microimmunofluorescence test

Treatment

Doxycycline or azithromycin

Prevention

- Erythromycin is effective in infected mothers to prevent neonatal disease.
- Treat neonatal conjunctivitis with systemic erythromycin to prevent pneumonia.

Chlamydia pneumoniae

- Respiratory infections. Atypical pneumonia (single lobe) is very common.
- Infect smooth muscle, endothelial cells of coronary artery and macrophages.
- Potential association with atherosclerosis.

Chlamydia psittaci

Distinguishing Characteristics

- *Chlamydia* associated with birds.
- No glycogen in the inclusion bodies.

Reservoir

Birds: parrots (psittacine), turkeys, others

Transmission

- Dust of dried bird secretions and feces
- Turkeys are a major U.S. reservoir.

Pathogenesis

Intracellular growth

Disease

Psittacosis (Atypical Pneumonia)

- **Pneumonia often occurs with hepatitis.** Fever, chills, rash, myalgia but generally mild/moderate while X-ray may look severe.
- Cough may be absent; when present, non-productive initially; then scant mucopurulent.

Laboratory Diagnosis

- Cytoplasmic inclusions seen on Giemsa or fluorescent-antibody-stained sputum or biopsy
- Organism can be isolated from sputum in tissue culture, but rarely done.
- Serodiagnosis by complement fixation test

Treatment

Doxycycline

Prevention

No vaccine or drug is available.

GENUS: *MYCOPLASMA*

- **Smallest free-living (extracellular) bacteria**
- **Missing peptidoglycan (no cell wall)**
- **Sterols in membrane**

Mycoplasma pneumoniae (Eaton's agent)

Distinguishing Characteristics

- Extracellular, tiny, flexible
- No cell wall. Not seen on Gram-stained smear
- Membrane with cholesterol but **does not synthesize cholesterol**
- Requires cholesterol for *in vitro* culture

Reservoir

Human respiratory tract

Transmission

Respiratory droplets; close contact: families, military recruits, medical school classes, college dorms

Pathogenesis

- Surface parasite: not invasive
- **Attaches to respiratory epithelium via P1 protein**
- **Inhibits ciliary action**
- **Produces hydrogen peroxide, superoxide radicals, and cytolytic enzymes,** which damage the respiratory epithelium, leading to necrosis and a bad hacking cough (walking pneumonia).

Diseases

Pneumonia

- **Pharyngitis**
- **May develop into an atypical pneumonia with persistent hack (little sputum produced)**
- **Most common pneumonia (along with viruses) in young adults**

Lab Diagnosis

- Primarily clinical diagnosis; PCR/nucleic acid probes
- Microscopy not useful
- **Fried egg colonies** on Mycoplasma or Eaton's media (have sterols); 10 days
- **Positive cold agglutinins** (autoantibody to red blood cells) test is not very specific and positive in only 65%.
- Complement fixation test for antibodies to *Mycoplasma pneumoniae* is more specific.

Treatment

Erythromycin, azithromycin, clarithromycin. **No cephalosporins nor penicillins.**

Prevention

None

Ureaplasma urealyticum

Distinguishing Characteristics

- Belongs to the ***Mycoplasma*** family
- **Produces a urease**

Diseases

- **Urethritis** (half of the non-Neisserial, non-Chlamydial), prostatitis
- Can cause renal calculi

Chapter Summary

The morphology of Gram-positive and Gram-negative bacteria is compared. Only the Gram-negative cells have an outer membrane and as a consequence a periplasmic space. The cell wall of Gram-positive cells is relatively thick and has teichoic acid filaments bound to it. Acid-fast cells contain mycolic acid in their cell walls.

The Gram stain is a four-step procedure. Cells are treated with crystal violet and then with Gram's iodine. Cells are then washed with acetone or alcohol, which removes the purple/blue stain from Gram-negative cells. Safranin is used as a counterstain, leaving Gram-positive cells purple/blue but staining Gram-negative cells red/pink.

The Ziehl-Neelsen/Kinyoun acid-fast stain is used to identify cells that retain a carbol-fuchsin red dye after washing with acetone or alcohol. *Mycobacterium* and *Legionella micdadei* are acid fast, *Nocardia* is partially acid fast, and all other bacteria are not acid fast. Cysts of *Cryptosporidium* and *Isopora* are also acid fast.

In processing sputa for *Mycobacterium*, samples are first screened using an auramine-rhodamine fluorescent stain. Samples showing fluorescence are then stained with the acid-fast stain to confirm the presence of acid-fast bacteria.

The internal structure of bacterial cells includes the circular, intron- and histone–free, DNA-containing nucleoid region; 70S ribosomes, consisting of 50S and 30S subunits; various granules; and, in many cases, plasmids. Bacterial cells have no mitochondria, chloroplasts, or any other membrane-bound organelle.

Two Gram-positive genera, *Bacillus* and *Clostridium*, are endospore producers. These spores have no reproductive function but permit survival under adverse conditions.

Bacterial cells reproduce by binary fusion. When cultured, they initially enter a short lag phase with no cell division and then enter a logarithmic (exponential) growth phase, followed by a stationary and finally a death phase.

The types of culture media required for growth of the various microbes are summarized in a series of tables (Tables I-2-6, I-2-7, and I-2-8).

Children should be vaccinated against diphtheria, tetanus, and pertussis with either the DTP or the newer DTaP (acellular) vaccine and against *Haemophilus influenzae* type b with the HIB vaccine.

It is recommended that senior citizens get vaccinated against *Streptococcus pneumoniae*. Other vaccines with specialized uses include those against *Neisseria meningitidis*, *Salmonella typhi*, *Yersinia pestis*, *Bacillus anthracis*, and Bacille Calmette Guérin (BCG).

Table I-2-9 lists the Gram-positive pathogens, Table I-2-10 lists the non-Gram staining pathogens, and Table I-2-11 lists the Gram-negative pathogens.

The remainder of this chapter is an inventory of the various medically important pathogenic bacteria, arranged by genus. The diseases associated with each genus and the important properties for the various species in each genus are succinctly described.

Review Questions

1. Staphylococci are routinely differentiated from streptococci by

   A. Coagulase test
   B. Test with hydrogen peroxide
   C. Polymerase chain reaction
   D. Protein A immune assay
   E. Growth in 6.5% sodium chloride

2. An atherosclerotic 80-year-old man develops a pelvic abscess following a ruptured appendix. What is/are the most likely causative agent(s)?

   A. *Bacteroides* species and microaerophilic streptococci
   B. *Candida albicans*
   C. *Enterobacter aerogenes*
   D. *Haemophilus influenzae* Group B
   E. *Streptococcus viridans*

3. A 21-year-old student was seen by his family physician with complaints of pharyngitis. Examination of the pharynx revealed patchy erythema and exudates on the tonsillar pillars. Throat smear showed Gram-positive cocci in chains and Gram-negative diplococci. He admitted having been sexually active. What is the significance of the Gram stain smear in this case?

   A. It provides a rapid means of diagnosing the infection
   B. It should have been examined by an experienced microbiologist
   C. It is not useful as it is not possible to make a diagnosis this way
   D. It strongly suggests gonococcal pharyngitis
   E. It is evidence of infection with hemolytic streptococci and neisseriae

4. With an appropriately performed acid-fast staining procedure, *Staphylococcus epidermidis* will appear

   A. Blue
   B. Red
   C. Purple
   D. Colorless
   E. Brown

5. *Treponema pallidum* can be identified in a syphilitic lesion (either primary or secondary stage) by

   A. Culture on Fletcher's serum semi-solid medium
   B. Immunofluorescent stain of smear made from the active lesion
   C. Gram stain
   D. Special culture using hemoglobin and yeast extract
   E. Rapid plasma reagin (RPR) assay

6. Which of the following properties is shared by *Legionella pneumophila* and *Mycobacterium avium-intracellulare*, an atypical *Mycobacterium*?

   A. Common cause of venereal disease
   B. Acid fast
   C. Requiring iron and cysteine for growth
   D. Not readily transmitted from person to person
   E. Inability to grow in laboratory culture media

7. What laboratory test is most useful for diagnosis of Lyme disease?

   A. Blood culture on sheep blood agar plate
   B. Spinal fluid culture on Thayer-Martin agar
   C. Detection of IgM/IgG antibodies to the spirochete
   D. Detection of specific antibody to *Ixodes* tick
   E. Documentation of fever and arthritis

8. With which of the following diseases is strict isolation indicated for the hospitalized patient?

   A. Botulism
   B. *Y. pestis* pneumonia
   C. Pneumococcal pneumonia
   D. *Mycobacterium kansasii* pulmonary infection
   E. Cervical-facial actinomycosis

9. A previously healthy 5-month-old infant now with apparent upper body weakness including droopy eyes, head lag, drooling, and inability to sit unassisted. The most likely infectious form is

   A. Elementary body
   B. Reticulate body
   C. Endospore
   D. Exotoxin
   E. Vegetative cell

10. Sixteen residents in a retirement home have fever, malaise, and anorexia. These residents have taken their meals prepared by the same kitchen. Blood cultures from 11 of these residents grow *Salmonella typhi*. The primary reservoir of this organism is

    A. Hen's egg
    B. Dogs and cats
    C. Turkeys
    D. People
    E. Water

11. If a culture is inoculated to a density of 5×10^2 cells/ml at time 0 and has both a generation time and lag time of 10 minutes, how many cells/ml will there be at 40 minutes?

    A. 1.5×10^3
    B. 2×10^3
    C. 4×10^3
    D. 6×10^3
    E. 4×10^6

12. A 6-year-old girl crashed on a toboggan ride and complained of pain in the perineal area. Exam showed only bruising of the area. Two days later, she developed fever, prostration, discoloration of the buttock and blebs of the skin in the area. After admission to the hospital, she developed progressive involvement of the leg, thigh, and buttock with extension to the lower abdomen. She went into shock and died before surgery could be performed. At autopsy, a 1-inch piece of wood was found in the perineum, which had perforated the anus. The most likely causative agent

    A. Requires an elevated oxidation reduction potential
    B. Is a Gram-negative coccobacillus
    C. Is a marked lecithinase producer
    D. Is non-hemolytic on blood agar
    E. Is non-fermentative

13. A 71-year-old male was admitted from his extended care facility (nursing home) because of recent aggravation of an exfoliative skin condition that has plagued him for several years. He had been receiving a variety of topical antibiotic regimens over the last year or two. He now has a temperature of 38.9°C (102°F). The skin of upper chest, extremities, and neck shows erythema with diffuse epidermal peeling and many pustular lesions. Cultures obtained from these lesions were reported back from the laboratory as yielding a Gram-positive organism that is highly salt (NaCl) tolerant. What lab result is used to confirm the species of the causative agent?

    A. Bacitracin sensitivity
    B. Bile solubility
    C. Catalase production
    D. Coagulase production
    E. Optochin sensitivity

14. Eight of 10 family practice residents who had a potluck 4 days ago now have diarrhea with abdominal cramps, general malaise, and fever ranging from 37.5° to 38.7°C. Stools from three are blood tinged. Laboratory studies revealed the causative agent was a microaerophilic Gram-negative, curved rod with polar flagella often in pairs to give a "seagull" appearance. It grew on special media at 42°C. The original contamination probably was found in

    A. Poultry
    B. Improperly canned food
    C. Fried rice
    D. Fish
    E. Vegetables

15. In the screen for bacterial meningitis (most commonly a latex particle agglutination test), what chemical component are we searching for in the cerebrospinal fluid?

    A. Cellular proteins
    B. Endotoxin
    C. Immunoglobulins
    D. Polysaccharide
    E. Ribosomal RNA

16. What percentage of the time does tetracycline resistance accompany methicillin resistance in methicillin-resistant *Staphylococcus aureus*?

    A. Less than 5% of the time
    B. 5–24% of the time
    C. 25–49% of the time
    D. 50–74% of the time
    E. Greater than 75% of the time

17. The structure that is found in Gram-negative but not in Gram-positive bacteria is

    A. Capsule
    B. Cell wall
    C. Cytoplasmic membrane
    D. Endospore
    E. Outer membrane

18. Since prokaryotes do not possess mitochondria, oxidative phosphorylation and electron transport in these cells take place in association with the

    A. Polysaccharide gel layer
    B. Lipopolysaccharide layer
    C. Peptidoglycan layer
    D. Periplasmic space
    E. Lipoprotein bilayer

19. A 5-year-old child of an Eastern European immigrant family is brought to your pediatric clinic. The child is afebrile, but weak and exhausted from a week of paroxysmal coughing with inspiratory whoops, frequently associated with vomiting. The parents profess religious objections to childhood vaccinations, but permit withdrawal of a blood sample, which reveals a lymphocytosis of 44,000/mm^3. Production of lymphocytosis, insulin secretion, and histamine sensitization are all results of which attribute of this organism?

    A. Filamentous hemagglutinin
    B. Adenylate cyclase toxin
    C. Beta-hemolysin
    D. Anaerobic growth
    E. Pertussis toxin
    F. Tracheal cytotoxin
    G. Motility throughout the circulation

20. The earliest definitive diagnosis of shigellosis in the U.S. is routinely made by clinical findings and

A. Culture of the stool
B. Identification of Shiga toxin in stools
C. Positive blood cultures
D. Isolation of a sorbitol fermenting Enterobacteriaceae either from stool or blood
E. Demonstration of fecal PMNs
F. PCR for H antigens

21. Which of the following bacterial structures or products is notoriously anti-phagocytic?

A. Teichoic acid of *Streptococcus pyogenes*
B. Bound coagulase of *Staphylococcus aureus*
C. Lipopolysaccharide (LPS)
D. Pili of gonococci
E. Peptidoglycan of rough strains of pneumococci

22. What structure is most responsible for triggering Gram-negative shock?

A. Capsule
B. Heat shock proteins
C. Outer membrane
D. Periplasmic space
E. Peptidoglycan-teichoic acid fragments
F. Sex pili

23. Pneumococcal pneumonia or meningitis rarely occurs in the absence of what virulence factor?

A. Capsule
B. Heat shock proteins
C. Outer membrane
D. Periplasmic space
E. Peptidoglycan-teichoic acid fragments
F. Sex pili

24. What antigen is most useful in identifying nephritogenic strains of Group A streptococci which may induce glomerulonephritis?

A. Capsular antigen
B. Cell wall carbohydrates
C. M proteins
D. Outer membrane proteins
E. P protein
F. Teichoic acids

25. What toxin stimulates adenylate cyclase by catalyzing the transfer of ADP-ribose to the inhibitory subunit of the G protein?

    A. Pertussis toxin
    B. Cholera toxin
    C. Diphtheria toxin
    D. *Escherichia coli* labile toxin
    E. *Escherichia coli* verotoxin
    F. *Shigella dysenteriae* Shiga toxin

26. What toxin blocks the function of the inhibitory neurotransmitter at synapses in the spinal cord leading to spasm?

    A. *Bordetella pertussis* neurotoxin
    B. Cholera toxin
    C. *Clostridium botulinum* toxin
    D. Diphtheria toxin
    E. *Escherichia coli* verotoxin
    F. Shiga toxin
    G. Tetanus toxin

27. What toxin inhibits acetylcholine release at the neuromuscular junction?

    A. *Bordetella pertussis* neurotoxin
    B. Cholera toxin
    C. *Clostridium botulinum* toxin
    D. Diphtheria toxin
    E. *Escherichia coli* verotoxin
    F. Shiga toxin
    G. Tetanus toxin

28. What toxin inhibits protein synthesis in mammalian cells by catalyzing the ADP-ribosylation of the elongation factor 2 (EF2)?

    A. Pertussis toxin
    B. Cholera toxin
    C. Botulinum toxin
    D. Diphtheria toxin
    E. *Escherichia coli* verotoxin
    F. Shiga toxin
    G. Tetanus toxin

29. What toxin continually stimulates adenylate cyclase to overproduce cAMP by catalyzing the binding of ADP-ribose to the Gs protein leading to severe fluid loss?

A. Pertussis toxin
B. Cholera toxin
C. *Escherichia coli* stable toxin
D. *Escherichia coli* verotoxin
E. Shiga toxin
F. Tetanus toxin

30. Which organism grows best in low O_2 concentrations but requires oxygen?

A. *Bacteroides melaninogenicus* (*Prevotella melaninogenica*)
B. *Campylobacter jejuni*
C. *Escherichia coli*
D. *Mycobacterium leprae*
E. *Mycobacterium tuberculosis*

31. What is the major chemical component giving bacterial cells protection from osmotic damage?

A. Lipopolysaccharide-phospholipid
B. Peptidoglycan
C. Phospholipid
D. Polysaccharide
E. Protein
F. Teichoic acid

32. A 27-year-old female, returning home from her honeymoon, has developed urinary frequency, dysuria, and urgency. Her urine is grossly bloody. Which lab data are most likely to define the causative agent?

A. A Gram-negative diplococcus, which is oxidase positive but does not ferment maltose
B. A Gram-positive coccus, which is catalase positive and coagulase negative
C. An optochin-resistant, catalase-negative, Gram-positive coccus
D. A Gram-positive bacillus grown on a low oxidation-reduction medium
E. A Gram-negative bacterium capable of reducing nitrates to nitrites

33. Two days after eating a meal that included home-canned green beans, three people developed various degrees of visual problems, including double vision and difficulties focusing. Describe the Gram reaction of the organism most likely to be isolated from the left-over beans and lab findings, which will be used in its identification.

A. A Gram-positive coccus, which is catalase-positive and grows in a high salt environment
B. A Gram-positive aerobic bacillus, which sporulates
C. A Gram-positive coccus, which is catalase-negative and optochin-resistant
D. A Gram-positive bacillus grown on a low oxidation-reduction medium
E. A Gram-negative bacillus capable of reducing nitrates to nitrites

34. A 16-year-old has pneumonia with a dry, hacking cough. The X-ray pattern shows a light, diffuse infiltrative pattern. The most likely organism producing these symptoms is

    A. A non-Gram-staining bacterium requiring sterols
    B. A bacillus showing granules when stained with methylene blue
    C. A bacitracin-sensitive, catalase-negative Gram-positive coccus
    D. A coagulase positive, Gram-positive coccus in clusters; catalase positive
    E. A Gram-positive bacillus grown on a low oxidation-reduction medium

35. Because of cold growth of this fairly common animal fecal bacterium potentially contaminating some deli meats and soft cheeses, renal transplant patients should only have hot deli sandwiches. What is this causative agent of meningitis in transplant patients?

    A. *Brucella* spp
    B. *Francisella tularensis*
    C. *Leptospira interrogans*
    D. *Listeria monocytogenes*
    E. *Streptococcus pneumoniae*

36. A 55-year-old woman had her rheumatic heart valve replaced with a prosthetic valve. Six blood cultures became positive after 3 days of incubation. An optochin-resistant, catalase-negative Gram-positive coccus that was alpha-hemolytic was isolated. What was the most likely causative agent?

    A. *Candida albicans*
    B. *Pseudomonas aeruginosa*
    C. *Serratia marcescens*
    D. *Staphylococcus aureus*
    E. *Streptococcus pneumoniae*
    F. *Streptococcus pyogenes*
    G. *Streptococcus viridans*

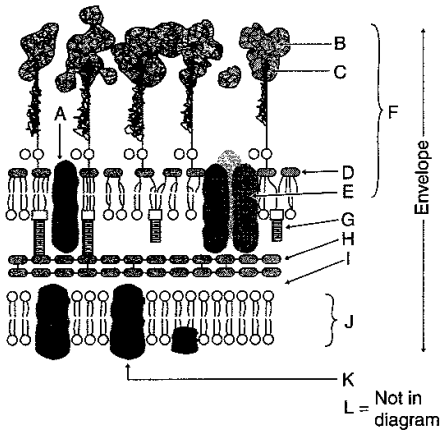
37. Which of the following organisms is killed by oxygen and ferments in absence of oxygen?

    A. *Bacteroides melaninogenicus* (*Prevotella melaninogenica*)
    B. *Campylobacter jejuni*
    C. *Escherichia coli*
    D. *Mycobacterium leprae*
    E. *Mycobacterium tuberculosis*

38. A virulence factor that causes *Mycobacterium tuberculosis* to clump together and grow in a "serpentine-like" fashion is

    A. Endotoxin
    B. M protein
    C. PPD (purified protein derivative)
    D. Slimy capsule
    E. Trehalose-6,6-dimycolate
    F. Wax D

39. From the diagram below, pick the structure that is associated with a passive transport across the membrane.



40. Calcium dipicolinate is found in

A. *Aspergillus*
B. *Bacillus*
C. *Escherichia*
D. *Mycobacterium*
E. *Rickettsia*
F. *Vibrio*

41. What are the trimeric structures involved in transport of materials across the outer membrane of the Gram-negative bacteria?

A. GTP-binding proteins
B. Lipopolysaccharides
C. Outer membrane proteins
D. Periplasmic space
E. Porin proteins
F. Prion proteins

42. What is the function of penicillin-binding proteins when there is no penicillin present in the bacterium's environment and the cell is actively replicating?

A. They are involved in microtubule formation and cell division
B. They have enzymatic activity: transpeptidases and carboxypeptidases
C. They are involved in protein elongation
D. They are involved in the supercoiling of DNA
E. They are transcriptional regulators

43. What is an organism called that respires in the presence of oxygen and ferments in the absence of oxygen?

    A. Aerobe
    B. Anaerobe
    C. Facultative aerobe
    D. Facultative anaerobe
    E. Microaerophile
    F. Obligate aerobe

44. A 15-day-old male presents with purulent conjunctivitis. Iodine staining bodies are seen in conjunctival scrapings. The most likely infectious form is

    A. Elementary body
    B. Reticulate body
    C. Endospore
    D. Exotoxin
    E. Vegetative cell
    F. Virus resistant to alcohol
    G. Virus sensitive to alcohol

45. What organism is most likely responsible for bacterial pneumonia in persons with alcohol intoxication?

    A. *Haemophilus influenzae*
    B. *Proteus vulgaris*
    C. *Pseudomonas aeruginosa*
    D. *Staphylococcus aureus*
    E. *Streptococcus pneumoniae*
    F. *Streptococcus viridans*

46. What organism is most likely responsible for bacterial meningitis in infants during the first month of life?

    A. *Enterococcus faecalis* (*Streptococcus faecalis*)
    B. *Haemophilus influenzae*
    C. *Staphylococcus aureus*
    D. *Streptococcus agalactiae*
    E. *Streptococcus pneumoniae*
    F. *Streptococcus pyogenes*

47. What organism is most likely responsible for bacterial endocarditis in men following urological instrumentation?

A. *Enterococcus faecalis*
B. *Pseudomonas aeruginosa*
C. *Streptococcus pyogenes*
D. *Streptococcus viridans*
E. *Ureaplasma urealyticum*

48. An AIDS patient with septicemia and a target-shaped necrotic lesion on the buttock with a black center and an erythematous margin. Which causative agent is most likely?

A. *Bacillus anthracis*
B. *Enterococcus faecalis*
C. *Pseudomonas aeruginosa*
D. *Staphylococcus aureus*
E. *Streptococcus pyogenes*

49. What causative agent is most likely responsible for edema, hematuria, proteinuria in a patient who had impetigo 3 weeks ago?

A. *Clostridium perfringens*
B. *Pseudomonas aeruginosa*
C. *Staphylococcus aureus*
D. *Staphylococcus epidermidis*
E. *Streptococcus agalactiae*
F. *Streptococcus pyogenes*

50. Patient was admitted to the hospital because of bleeding duodenal ulcer. Culture at 37°C grew urease-positive curved bacteria. The most likely causative agent is

A. *Campylobacter jejuni*
B. *Entamoeba histolytica*
C. *Enterococcus faecalis* (*Streptococcus faecalis*)
D. *Helicobacter pylori*
E. *Pseudomonas aeruginosa*

Answers

1. **Answer: B.** The catalase test is carried out with hydrogen peroxide. The other four tests do not differentiate.

2. **Answer: A.** Atherosclerosis leads to poor circulation to the lower extremities, which in turn lowers the oxidation-reduction potential of the tissues. All this predisposes to infections caused by anaerobic M-Os, in this case, *Bacteroides* and *Streptococci.* The patient is suffering from anaerobic cellulitis or possibly myonecrosis.

3. **Answer: C.** Gram-positive cocci (alpha hem. Strep) and Gram-negative cocci (neisseriae) are normally present in the throat. There is no way to differentiate pathogens from nonpathogens by the Gram stain.

4. **Answer: A.** Students need to remember that the primary stain in the acid-fast stain is carbol-fuchsin, which stains acid-fast organisms red. The counterstain is methylene blue, which stains everything else blue. They also need to know the three important genera of acid-fast organisms: *Mycobacterium, Nocardia,* and *Cryptosporidium.*

5. **Answer: B.** *Treponema* cannot be cultured. Fletcher's medium is for *Leptospira.* The spirochete that will take Gram stain is *Borrelia.* RPR is for detection of antibody.

6. **Answer: D.** Neither one is common for venereal disease. *Legionella* is not acid fast. Iron and cysteine requirement refers to *Legionella.* Both can grow in artificial media.

7. **Answer: C.** *Borrelia burgdorferi,* the causative agent of Lyme disease, grows in a complex medium, not on sheep blood agar. Thayer-Martin is for pathogenic neisseriae. There is no purpose to detect antibody to the tick. ELISA or some rapid test for IgG/IgM is currently used for diagnosis of Lyme disease.

8. **Answer: B.** *Y. pestis* causes bubonic plague, which is not contagious through respiratory droplets; however, the bubonic form can progress via septic emboli to pneumonia, which is contagious. Adult botulism is a toxemia, thereby not contagious. Pneumococcal pneumonia is caused by *S. pneumoniae,* which is not considered highly infectious and colonizes many people without causing disease. Choice D (*Mycobacterium kansasii*) and choice E (*Actinomyces*) are both environmental M-O: not contagious.

   Remember that as a rule-of-thumb, M-O that have the environment (water or soil) as reservoirs, and those that are zoonotic or arthropod-borne, and those that are normal human flora are generally not contagious from person to person. *Y. pestis* is an exception.

9. **Answer: C.** Infant botulism is a toxi-infection started by the ingestion of *Clostridium botulinum* endospores from the environment. The spores geminate in the immature flora of the GI tract and the toxin is produced *in vivo* in contrast to adult botulism where the preformed toxin is ingested.

10. **Answer: D.** The reservoir for *S. typhi* is people (humans). Other species of *Salmonella* have animals as their reservoirs.

11. **Answer: C.** Explanation: Remember that each cell divides into two at each generation following the single lag phase. So at the end of the first 10 minutes there is still 5×10^2, and then at the end of the first 20 minutes (total) there are 10×10^2. At the end of 30 minutes total time there will be 20×10^2, and at the end of the total time, 40×10^2, which is written 4×10^3 in proper scientific notation.

12. **Answer: C.** The description suggests strongly that she has myonecrosis. Therefore, the causative agent (at least one) is *C. perfringens. C. perfringens* is an anaerobe, therefore choice A is wrong. Clostridia are all Gram-positive, therefore choice B is wrong. *C. perfringens* have concentric areas of beta hemolysis, therefore choice D is wrong. *C. perfringens* is a marked lecithinase producer; therefore choice C is correct.

13. **Answer: D.** The patient has the "scalded skin" syndrome caused by *S. aureus.* The GENUS *Staphylococcus* would be distinguished from Strep by Staphylococcal production of catalase. But the SPECIES (*S. aureus*) would be distinguished from *S. epidermidis* on the basis of *S. aureus* production of coagulase. Bacitracin sensitivity and bile solubility are species characteristics of Strep pneumoniae.

14. **Answer: A.** The clue is Gram-negative curved rods with polar flagella often in pairs to give a "seagull" appearance and the microaerophilic on special media and growing at 42°C. That description is most compatible with *Campylobacter jejuni.* Poultry are one of the most important reservoirs so choice A is the correct response.

15. **Answer: D.** The diagnostic test looks for capsular material, which is a polysaccharide (choice D). The only non-polysaccharide one is anthrax, which is a polypeptide. Immunoglobulins would not be found this early in the CSF and may never be formed in some severely immunocompromised patients.

16. **Answer: E.** Methicillin-resistant strains of *Staph aureus* are generally resistant to all available antibiotics except for vancomycin, teicoplanin, and fusidic acid. Therefore, the answer is generally 100%, making choice E the best answer.

17. **Answer: E.** Capsules, cell wall, and cytoplasmic membranes are found in both Gram-positive and Gram-negative bacteria. Endospores (choice D) occur with certain Gram-positive bacteria, e.g., *Bacillus* and *Clostridium.* Only Gram-negatives have an outer membrane.

18. **Answer: E.** The site of most metabolic processes in the prokaryotic cell is the cytoplasmic membrane, which is best described as a lipoprotein bilayer (choice E). Of the distractors: Polysaccharide (choice A) describes the capsule layer, which is antigenic and antiphagocytic and outside the cell; lipoprotein (choice B) describes the outer membrane of Gram-negative bacteria, which also is basically outside of the cell; peptidoglycan (choice C) refers to the cell wall layer of all bacteria and periplasmic space; choice D refers to the space between the inner membrane and cell wall of Gram-negative bacteria. None of the distractors (A)–(D) are significant sites of action of any of the metabolic enzymes.

19. **Answer: E.** The disease here is whooping cough, caused by *Bordetella pertussis.* The pertussis toxin (also known as the lymphocytosis-promoting toxin) is not believed to be directly cytotoxic, but stimulates adenylate cyclase by ribosylating regulatory proteins. It causes a variety of effects depending on the cell type involved: insulin secretion, lymphocytosis, and alteration of immune effector cells. Of the distractors: the filamentous hemagglutinin (choice A) mediates attachment; the adenylate cyclase toxin (choice B) stimulates local edema; the organism produces only a small zone of hemolysis around its colonies, so choice C is not true; it is an aerobe and does not grow anaerobically (choice D); and is nonmotile (choice G). All systemic manifestations of the disease arise from the circulation of the toxins, not the organism itself.

20. **Answer: A.** Shigellosis is an invasive disease confined to the intestine. Definitive diagnosis is made by prompt stool culture (usually positive by week 2). The finding of PMNs is not definitive for *Shigella.* In the U.S., the most common organism is *Shigella sonnei* or *flexneri,* neither of which produce Shiga toxin, making choice B false. Although *Shigella* is invasive, it does not invade the vasculature so blood cultures would not be positive. Sorbitol fermentation is used in isolation of most strains of VTEC. Remember that Shigellae are nonmotile; thus, there are no H antigens.

21. **Answer: D.** The correct answer to this question is the pili of *Neisseria gonorrhoeae.* Another antiphagocytic component is any bacterial capsule (missing in rough strains of *Pneumococcus* and the peptidoglycan does not inhibit phagocytosis, making choice E not a proper choice). Other correct answers would have been M-protein of *Strep pyogenes,* and the A proteins of *Staph aureus.*

22. **Answer: C.** Lipid A is the actual component of LPS, which is responsible for triggering Gram-negative shock. Since it is not listed, then you need to think about where it is found as it is a structural toxin. It is located in the outer membrane, and thus this is the correct answer. Peptidoglycan-teichoic acid fragments are found in Gram-positive bacteria only and are responsible for triggering septic shock when a Gram-positive organism is in the bloodstream, or inflammatory response when it is in the CSF or in tissues.

23. **Answer: A.** The most important virulence factor for *S. pneumoniae* is the capsule. Without it the strains are avirulent, except in seriously immunocompromised patients.

24. **Answer: C.** Remember first that Group A Strep is also called *Streptococcus pyogenes* and that it has a nonimmunogenic capsule of hyaluronic acid. The important antigen here is a surface protein called the M protein; it is used in "typing" GAS. Certain M types are more commonly involved in glomerulonephritis with M12 the most common in AGN. The large number of M-protein types and nonimmunogenic capsules (hyaluronic acid) are why many people get repeated Strep infections.

25. **Answer: A.** Pertussis toxin activates adenylate cyclase by turning off G_i protein via ADP-ribosylation. Cholera toxin and the labile toxin of *E. coli* are the other two that ADP ribosylate G proteins, but their ADP ribosylation is of G_S. Both verotoxin and Shiga toxin clip the 60S ribosomal subunit.

26. **Answer: G.** Tetanus toxin blocks the release of inhibitory factor (glycine) at the spinal cord. If one forgets the mode of action, think of the clinical presentation of tetanus, which is spasm rather than paralysis, as in botulism.

27. **Answer: C.** This is the mode of action of botulism toxin, and the result is paralysis of muscles.

28. **Answer: D.** Diphtheria toxin inactivates EF2 and thus inhibits protein synthesis. Students need to remember *Pseudomonas* exotoxin will do the same—a question frequenting USMLE exams.

29. **Answer: B.** Cholera toxin locks G_S protein in the "on" position via ADP-ribosylation. Adenylate cyclase is thus continually being stimulated. Most severe fluid loss leading to hypovolemic shock occurs with cholera.

30. **Answer: B.** *Campylobacter* is the only microaerophile on the list.

31. **Answer: B.** Although the outer membrane (which is chemically a lipopolysaccharide-phospholipid) plays a minor role in protecting Gram-negative bacteria from osmotic damage, it is not as important as the cell wall. CW is the peptidoglycan net structure that offers major protection from osmotic damage and confers cell shape.

32. **Answer: E.** *E. coli*-induced cystitis is highest in sexually active females. It generally reduces nitrates and is also a lactose fermenter. Choice A = *Neisseria gonorrhoeae;* choice B = *Staphylococcus saprophyticus;* choice C = *Enterococcus faecalis* is one possibility; choice D = *Clostridium.*

33. **Answer: D.** This case history describes botulism (key words: home-canned green beans and visual problems). Foods classically associated are those with a neutral or alkaline pH. *C. botulinum,* the agent of botulism, is an anaerobe and thus has a low oxidation-reduction requirement. The other Gram-positive bacillus (aerobic) would be *Bacillus cereus.* Choice A = *Staph aureus;* choice B = *Bacillus cereus;* choice C = *S. viridans;* choice D = *Clost botulinum;* choice E = *E. coli.*

34. **Answer: A.** The disease is most likely mycoplasma pneumonia caused by *Mycoplasma pneumoniae,* which is non-Gram staining and requires cholesterol for growth. Choice B = *C. diphtheriae;* choice C = *S. viridans;* choice D = Staph aureus; choice E = *Clostridium.*

35. **Answer: D.** Meningitis in renal transplant patients and many cancer patients is frequently *Listeria* acquired from foods. This is also a problem if pregnant women are infected; it may cause meningitis in neonates.

36. **Answer: G.** A patient with a history of rheumatic fever and blood cultures growing an alpha hemolytic coccus, which is catalase-positive and optochin-resistant.

37. **Answer: A.** This is one of the dark-pigmented *Bacteroides,* now called *Prevotella.* They are obligate anaerobes.

38. **Answer: E.** Cord factor (trehalose dimycolate) causes serpentine-like clumps of TB organism.

39. **Answer: E.** This refers to the porins that passively (with the aid of electrostatic charges) allow entry of materials into the periplasmic space, from whence they are actively transported across the cytoplasmic membrane.

40. **Answer: B.** Calcium dipicolinate is found only in the core of bacterial spores. It plays a role in the dehydration and stabilization of the DNA in the spores. The two bacterial genera that form endospores are *Bacillus* and *Clostridium.*

41. **Answer: E.** The trimeric structures spanning the outer membrane are porins, a series of proteins forming a pore, permitting passive transfer of materials into the periplasmic space. They are outer membrane proteins. Please remember that this is a "best answer test," and if an answer is more specific to the question, it would be better.

42. **Answer: B.** Penicillin-binding proteins (PBPs) are involved in the final cross linkage of new pieces of peptidoglycan. PBPs are cellular transpeptidases and carboxypeptidases. They are located in the cytoplasmic membrane.

43. **Answer: D.** This describes a facultative anaerobe. Facultative aerobe is a term that does not exist.

44. **Answer: A.** The patient has inclusion conjunctivitis caused by *Chlamydia trachomatis.* The only form of this bacterium that has the ability to bind to the membranes and infect is the elementary form.

45. **Answer: E.** *S. pneumoniae* is the most common cause of community-acquired pneumonia. One of the predisposing factors is alcohol intoxication; another one is influenza virus infection.

46. **Answer: D.** *Strep agalactiae* is one of the most prominent neonatal pathogens. The other common causative agent is *E. coli,* which is not on the list.

47. **Answer: A.** Several organisms on this list can cause endocarditis under various conditions. *E. faecalis* is the one associated with urological manipulation. *S. viridans* is associated with oral manipulation.

48. **Answer: C.** *Pseudomonas aeruginosa.* Target-shaped necrotic lesion with a black center and an erythematous margin depicts ecthyma gangrenosum caused by *Pseudomonas* associated with immunodeficiency.

49. **Answer: F.** Edema, hematuria, and proteinuria are pathognomonic for acute glomerulonephritis following *S. pyogenes* skin infections. Impetigo can be caused by *S. aureus* and *S. pyogenes* but only *S. pyogenes* leads to acute glomerulonephritis.

50. **Answer: D.** This patient had *Helicobacter,* which is urease positive. *Campylobacter jejuni* also grows at 42°C, but it is urease negative.

Medically Important Fungi

3

What the USMLE Requires You to Know

- Basic morphology of fungi (hyphae, yeast, dimorphic, and various types of conidia)
- Basic chemistry, particularly that involved in antifungals or that distinguishes fungi from other groups
- Scientific names of the fungal pathogens and opportunists found in the United States
  - What are the four dimorphic fungi?
  - Recognize their tissue and environmental forms.
  - What are the three dermatophytes and what tissues do they invade?
  - What is the most common cause of meningitis in AIDS patients?
  - What does it look like? How do you diagnose?
  - What is a common cause of interstitial pneumonitis in AIDS patients?
  - Why is it considered a fungus?
  - Which medically important fungus has a capsule?
  - Which is found most commonly inside cells of the reticuloendothelial system?
- The diseases they cause and:
  - How acquired (geography, route)
  - Common presenting symptoms, most common sites of dissemination (if they disseminate commonly)
  - What two fungi are a problem in IV lines?
  - Most common cause of fungal septicemia and the clues used in the cases (germ tube test positive, pseudohyphae and true hyphae as well as yeast forms)

MYCOLOGY

Mycology is the **study of fungi (molds, yeasts, and mushrooms).**

All fungi are

- **Eukaryotic** (e.g., true nucleus, 80S ribosomes, mitochondria, as are humans).
- **Complex carbohydrate cell walls: chitin, glucan, and mannan.**
- **Ergosterol = Major membrane sterol**
  Imidazole antifungals inhibit synthesis of ergosterol.
  Polyene antifungals bind more tightly to **ergosterol** than cholesterol.
- Heterotrophic (require organic carbon)
  - Saprophytic or saprobic (fungus living on dead organic material)
  - Parasitic (fungus living on another living organism)

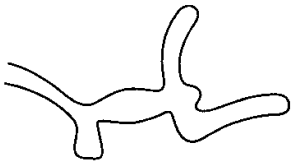


Figure I-3-1.
Nonseptate Hyphae

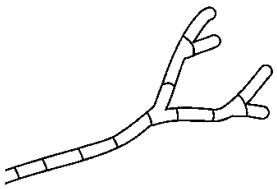


Figure I-3-2.
Septate Hyphae

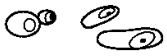


Figure I-3-3. Yeasts

FUNGAL MORPHOLOGY

Hyphae = filamentous cellular units of molds and mushrooms

Nonseptate Hyphae
- No cross walls
- Broad hyphae with **irregular width**
- **Broad angle of branching**

Septate Hyphae
- With **cross walls**
- Width is fairly regular (tube-like).

Hyphal Coloration
- **Dematiaceous: dark colored** (gray, olive)
- **Hyaline: clear**

Mat of hyphae = mycelium

Yeasts = single celled (round to oval) fungi

Dimorphic Fungi
- **Fungi able to convert from hyphal to yeast or yeast-like forms.**
- **Thermally dimorphic: in the "cold" are the mold form.**

Key Dimorphic Fungi

Histoplasma
Blastomyces
Coccidioides
Sporothrix

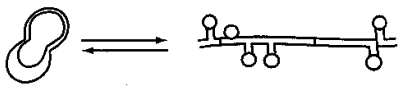


Figure I-3-4. Dimorphic Fungi

Pseudohyphae (Candida albicans)

Hyphae with constrictions at each septum

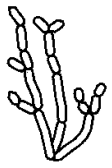


Figure I-3-5. Candida
Pseudohyphae

Spore Types

Conidia
- **Asexual spores**
- Formed off of hyphae
- Common
- Airborne

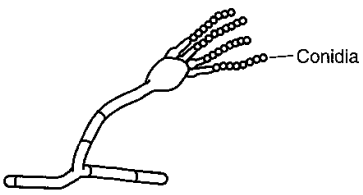


Figure I-3-6. Conidia

Blastoconidia: "Buds" on yeasts (asexual budding daughter yeast cells)

Figure I-3-7. Blastoconidia

Arthroconidia: Asexual spores formed by a **"joint"**

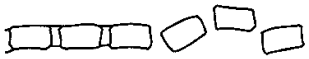


Figure I-3-8. Arthroconidia

Spherules and Endospores (***Coccidioides***): Spores inside the spherules in tissues

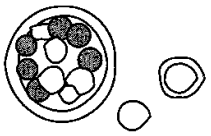
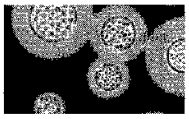


Figure I-3-9. Endospores and Spherules

Diagnosis

Table I-3-1. Microscopic Methods/Special Fungal Stains

Preparation	Fungal Color	Notes
KOH wet mount (KOH degrades human tissues releasing hyphae and yeasts)	Colorless (hyaline) refractive green or light olive to brown (dematiaceous) fungal elements	Heat gently; let set 10 minutes; dissolves human cells
PAS	Hot pink	
Silver stain	Old rose gray to black	
Calcofluor white (Can be done on wet mounts.)	Bright blue-white on black	Scrapings or sections, fluorescent microscope needed
India ink wet mount of CSF sediment	Colorless cells with halos (capsule) on a black particulate background (*Cryptococcus neoformans*)	Only "rules in." Insensitive; misses 50%.  **Figure I-3-10. *Cryptococcus neoformans***

Culture

(May take several weeks.) Special fungal media: inhibitory mold agar is modification of Sabouraud's with antibiotics.

- **Sabouraud's agar**
- Blood agar
- Both of the above with antibiotics

Identification from Cultures

- Fungal **morphology**
- PCR with nucleic acid probes

Serology

(E.g., antibody screen, complement fixation, etc.) Looking for patient antibody.

Fungal Antigen Detection: (CSF, serum)

Cryptococcal capsular polysaccharide detection by latex particle agglutination (LPA) or counter immunoelectrophoresis

Skin Tests

- Most useful for **epidemiology** or **demonstration of anergy** to an agent you know patient is infected with (grave prognosis)
- Otherwise, like tuberculosis, a skin test **only indicates exposure** to the agent.

NONSYSTEMIC FUNGAL INFECTIONS

Superficial Infections (Keratinized Tissues)

Malassezia furfur (Fungus Name)

Normal skin flora (lipophilic yeast)

Diseases

- **Pityriasis or tinea versicolor**
  - Superficial infection of keratinized cells
  - **Hypopigmented spots on the chest/back** (blotchy suntan)
  - KOH mount of skin scales: spaghetti and meatballs **Yeast clusters & short curved septate hyphae**
  - Treatment is topical selenium sulfide; recurs.
- **Fungemia in premature infants** on intravenous lipid supplements

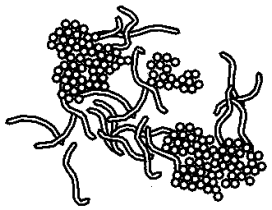


Figure I-3-11.
Malassezia furfur

Cutaneous Fungal Infections (without systemic disease)

Yeast or dermatophytic infections.

Yeast Skin Infections

- Commonly **cutaneous or mucocutaneous candidiasis**
- May disseminate in compromised patients
- Discussed with opportunistic fungi

Dermatophytes (Group of Fungi)

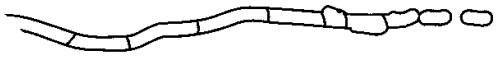


Figure I-3-12. Dermatophyte

- **Filamentous** fungi (**monomorphic**)
- **Infect only skin and hair and/or nails** (do not disseminate)
- Three genera:

  Trichophyton **- Infects skin, hair and nails**
  Microsporum **- Infects hair and skin**
  Epidermophyton **- Infects nail and skin**

Diseases

Dermatophytic Infections = **Tineas** (Ringworms)

- If **highly inflammatory**, generally from **animals** (zoophilic) (i.e., ***Microsporum canis***: cats or dogs)
- If **little inflammation**, generally from humans (**anthropophilic** tinea capitis: ***M. audouinii***)
- Tinea capitis = ringworm of the scalp
- The most serious of the tineas capitis is **favus** (tinea favosa), which causes **permanent hair loss** and is very contagious.
- Tinea barbae = ringworm of the bearded region
- Tinea corporis = dermatophytic infection of the glabrous skin
- Tinea cruris = jock itch
- Tinea pedis = athlete's foot

Diagnosis

- *Microsporum* fluoresces (Wood's lamp)
- **KOH mount** of nail or skin scrapings should show **arthroconidia and hyphae.**

Treatment

- Topical imidazoles or tolnaftate
- Oral imidazoles or griseofulvin where hairs are infected, or skin contact hurts
- Keep areas dry.
- **ID reaction (Dermatophytid) = Allergic response to circulating fungal antigens**

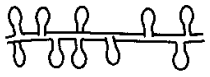


Figure I-3-13.
Sporothrix Hyphae

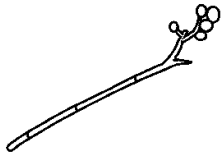


Figure I-3-14.
Sporothrix

Subcutaneous Mycoses

Sporothrix schenckii

Dimorphic Fungus

- **Environmental form:** on **plant material**, world wide as **hyphae with rosettes and sleeves of conidia**
- **Traumatic implantation** (rose or plum tree thorns, wire/sphagnum moss)
- **Tissue form: cigar-shaped yeast** in tissue

Diseases

- **Sporotrichosis (rose gardener's disease): subcutaneous or lymphocutaneous lesions.** Treatment: itraconazole or **potassium iodide in milk**
- **Pulmonary** (acute or chronic) **sporotrichosis.** Urban alcoholics, particularly homeless (**alcoholic rose-garden-sleeper's disease**)

DEEP FUNGAL INFECTIONS

Classical Pathogens

Three important classical pathogens in the U.S.A.:

Histoplasma

Coccidioides

Blastomyces

All three cause

- **Acute pulmonary** (asymptomatic or self-resolving in about **95%** of the cases)
- **Chronic** pulmonary, or
- **Disseminated** infections

Diagnosis

(Most people never see a doctor.)

- Sputum cytology (calcofluor white helpful)
- **Sputum cultures** on blood agar and **special fungal media** (inhibitory mold agar, Sabouraud's)
- **Peripheral blood cultures are useful for *Histoplasma* since it circulates in RES cells.**

Histoplasma capsulatum

Dimorphic Fungus

- **Environmental form: hyphae** with **microconidia** and **tuberculate macroconidia**
  - Endemic region: **Eastern Great Lakes, Ohio, Mississippi, and Missouri River beds**

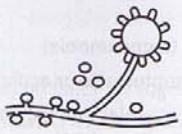


Figure I-3-15. *Histoplasma* Environmental Form

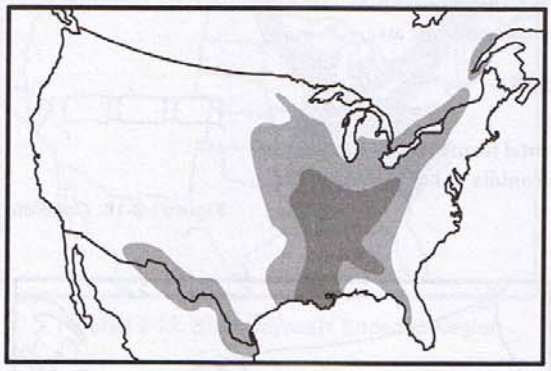


Figure I-3-16. *Histoplasma* Endemic Region

  - Found in **soil (dust) enriched with bird or bat feces**
  - Spelunking (cave exploring), cleaning chicken coops, or bulldozing starling roosts
- **Tissue form: small intracellular yeasts** with narrow neck on bud; **no capsule**
- **Facultative intracellular parasite** found in **reticuloendothelial (RES) cells** (tiny; can get 30 or so in a human cell)

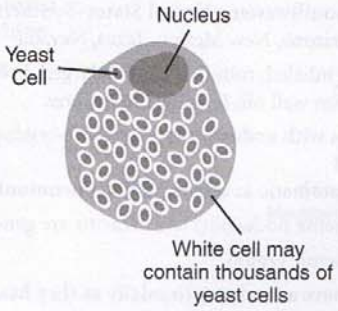


Figure I-3-17. Human RES Cell

Disease

Fungus flu (a pneumonia)

- Asymptomatic or acute (but self-resolving) pneumonia with flu-like symptomatology
- **Hepatosplenomegaly may be present** even in acute pulmonary infections (facultative intracellular RES)
- Very common in summer in endemic areas: kids or newcomers (80% of adults are skin-test positive in some areas)
- Lesions have a tendency to **calcify as they heal.**
- Relapse potential increases with T cell immunosuppression.
- **Disseminated infections:** Mucocutaneous lesions are common; also common **in AIDS** patients in endemic area.

Coccidioides immitis

Dimorphic Fungus

- **Environmental form: hyphae** breaking up into **arthroconidia** found **in desert sand.**

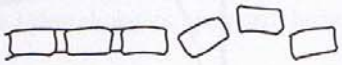


Figure I-3-18. *Coccidioides immitis*

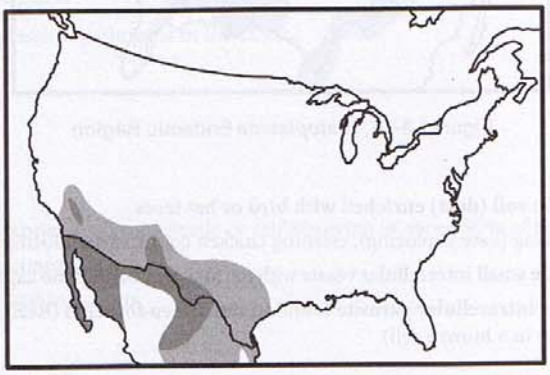


Figure I-3-19. *Coccidioides* Endemic Region

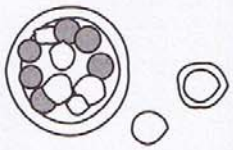


Figure I-3-20. *Coccidioides immitis* Spherules

  - Endemic region: **Southwestern United States**–Southern **California** (especially San Joaquin Valley), Arizona, New Mexico, Texas, Nevada
  - Arthroconidia are inhaled, round up, and enlarged, becoming spherules inside which the cytoplasm wall off, forming endospores.
- **Tissue form: spherules with endospores**

Disease: Valley Fever (asymptomatic to **self-resolving pneumonia**)

- **Desert bumps** (erythema nodosum) and arthritis are generally good prognostic signs.
- Very common in endemic region
- **Pulmonary lesions have a tendency to calcify as they heal.**
- **Systemic infections are a problem in AIDS and immunocompromised patients** in endemic region (meningitis, mucocutaneous lesion).
  - Cocci has a tendency to **disseminate in third trimester of pregnancy.**

Blastomyces dermatitidis

Dimorphic Fungus

Environmental form: hyphae with **nondescript conidia** (i.e., no fancy arrangements)

- Association not definitively known, **appears to be associated with rotting wood** such as beaver dams
- Endemic region: Upper Great Lakes, Ohio, Mississippi River beds plus the southeastern seaboard of the U.S. and northern Minnesota into Canada

Figure I-3-22. Blastomycosis Endemic Region

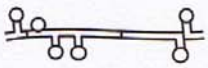


Figure I-3-21.
Blastomyces dermatitidis
Hyphae with Conidia

Tissue form: broad-based budding yeasts and a double refractile cell wall (not capsule)

Disease: Blastomycosis

- Acute and chronic pulmonary disease
- Considered less likely to self-resolve than *Histoplasma* or *Coccidioides*, so many physicians will treat even acute infections with ketoconazole.
- Disseminated disease

Figure I-3-23.
Blastomyces dermatitidis
Broad-Based Budding Yeasts

Opportunistic Fungi

Aspergillus fumigatus

Monomorphic filamentous fungus

- **Dichotomously branching**
- **Generally acute angles**
- **Septate**
- One of our **major recyclers**: compost pits, moldy marijuana

Figure I-3-24.
***Aspergillus* Showing**
Monomorphic Filamentous
Fungus

Diseases/Predisposing Conditions

- **Allergic bronchopulmonary aspergillosis**/asthma, allergies (growing in mucous plugs in the lung but not penetrating the lung tissue)
- **Fungus ball**: free in preformed lung cavities (surgical removal to reduce coughing, which may induce pulmonary hemorrhage)
- **Invasive aspergillosis**/severe neutropenia, CGD, CF, burns
  Invades tissues causing infarcts and hemorrhage.
  Nasal colonization → **pneumonia or meningitis**
  Cellulitis/in burn patients; may also disseminate.

Treatment

- Depends on severity of disease and underlying conditions: Itraconazole or amphotericin B

Candida albicans (and other species of *Candida*)

- **Yeast** endogenous to our mucous membrane normal flora
- *C. albicans* yeasts form germ tubes at 37°C in serum.
- Form **pseudohyphae** and **true hyphae** when it invades tissues (nonpathogenic *Candida* do not).

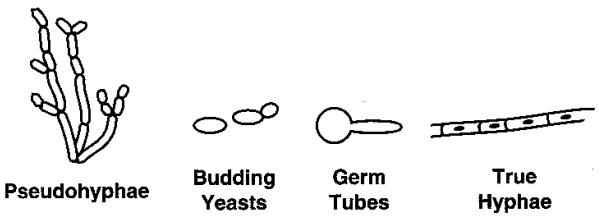


Figure I-3-25. *Candida albicans*

Diseases/Predisposing Conditions

- **Perlèche**: crevices of mouth/malnutrition
- **Oral thrush**/prematurity, antibiotic use, immunocompromised (IC) host, AIDS
- **Esophagitis**/antibiotic use, IC host, AIDS
- **Gastritis**/antibiotic use, IC host, AIDS
- **Septicemia** (with endophthalmitis and macronodular skin lesions)/immunocompromised, cancer and intravenous (IV) patients
- **Endocarditis** (with transient septicemias)/**IV drug abusers**
- **Cutaneous infections**/obesity and infants; patients with rubber gloves
- **Yeast vaginitis**/particularly a problem in diabetic women
- Chronic mucocutaneous candidiasis/endocrine defects; anergy to *Candida*

Diagnosis

- KOH: pseudohyphae, true hyphae, budding yeasts
- Septicemia: culture lab identification: biochemical tests/formation of germ tubes

Treatment

- Topical imidazoles or oral imidazoles; nystatin
- Disseminated: Amphotericin B or fluconazole

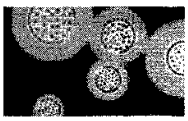


Figure I-3-26. *Cryptococcus neoformans*

Cryptococcus neoformans

Encapsulated Yeast (Monomorphic)

Environmental Source: Soil enriched with pigeon droppings

Diseases/Predisposing Conditions

- **Meningitis/Hodgkin's, AIDS (the dominant meningitis)**
- **Acute pulmonary** (usually asymptomatic)**/pigeon breeders**

Diagnosis of Meningitis: CSF

- Detect capsular antigen in CSF (by latex particle agglutination or counter immuno-electrophoresis)
- India ink mount (misses 50%) of CSF sediment to find budding yeasts with capsular "halos"
- Cultures (urease positive yeast)

Treatment: AMB+5FC until afebrile and culture negative, then fluconazole

Mucor, Rhizopus, Absidia (Zygomycophyta)

Nonseptate filamentous fungi

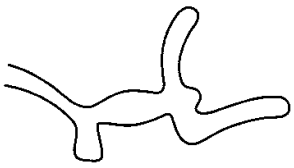


Figure I-3-27. Nonseptate Hyphae with Broad Angles

Environmental Source: Soil; sporangiospores are inhaled

Disease

- **Rhinocerebral infection** caused by *Mucor* (or other zygomycophyta)
- (Old names: Mucormycosis = Phycomycosis = Zygomycosis)
- Characterized by paranasal swelling, necrotic tissues, hemorrhagic exudates from nose and eyes, and mental lethargy
- Occurs in **ketoacidotic diabetic patients and leukemic patients.**
- These fungi penetrate without respect to anatomical barriers, progressing rapidly from sinuses into the brain tissue.

Diagnosis: KOH of tissue; broad ribbon-like nonseptate hyphae with about 90° angles on branches.

Treatment

- Debride necrotic tissue and start Amphotericin B fast
- High fatality rate because of rapid growth and invasion

Pneumocystis carinii

Fungus (based on molecular techniques like **ribotyping**)

- **Obligate extracellular parasite**
- **Silver stained cysts in tissues**

Figure I-3-28. *Pneumocystis*

Disease: Interstitial pneumonia

- Pneumonia in AIDS patients even with prophylaxis (mean CD4+/mm^3 of 26), malnourished babies, premature neonates, and some other IC adults and kids
- Symptoms: fever, cough, shortness of breath; sputum nonproductive except in smokers
- *Pneumocystis* attaches to and kills Type I pneumocytes, causing excess replication of Type II pneumocytes and damage to alveolar epithelium. Serum leaks into alveoli, producing an exudate with a foamy or **honeycomb appearance on H & E stain.** (Silver stain reveals the holes in the exudate are actually the cysts and trophozoites, which do not stain with H & E.)
- X-ray: **patchy infiltrative** (ground glass appearance), the lower lobe periphery may be spared.

Diagnosis: Silver-staining cysts in bronchial alveolar lavage fluids or biopsy

Treatment: Trimethoprim/sulfamethoxazole

Chapter Summary

Mycology is the study of fungi. The fungi include molds, yeasts, and mushrooms. All fungi are eukaryotic, have complex cell walls, have ergosterol in their cell walls, and are heterotrophic, either as free-living saprophytes or as parasites.

Yeasts are single-celled fungi. Some fungi grow as extended filamentous units called hyphae and can be classified according to the presence (septate-types) or absence (nonseptate-types) of cross walls and color. A mat of hyphae is called a mycelium.

Dimorphic fungi can convert between hyphal and yeast forms. Important examples of such fungi are *Histoplasma, Blastomyces, Coccidioides,* and *Sporothrix.*

Pseudohyphal forms present in *Candida albicans* have constrictions in the hyphae that resemble budding yeasts.

Most fungi reproduce by forming asexual spores called conidia. Variant forms of conidia include blastoconidia and arthroconidia. Some form sexual endospores inside of spherules in host tissues.

Cutaneous fungi are generally diagnosed by using a KOH scraping of skin. Other fungal preparations also may be stained using periodic acid-Schiff (PAS) stain, silver stain, calcofluor, or India ink.

Fungi may also be cultured on Sabouraud's or blood agar with antibiotics and then identified by cell morphology or by polymerase chain reaction (PCR) using specific nucleic acid probes.

Nonsystemic fungal infections include pityriasis (tinea) versicolor or fungemia in premature infants, caused by *Malassezia furfur;* cutaneous or mucocutaneous candidiasis; and nail and hair infections caused by species of *Trichophyton, Microsporum,* or *Epidermophyton.*

Sporotrichosis is a subcutaneous or lymphatic (rose gardener's disease) or pulmonary (alcoholic rose-garden-sleeper's disease) mycosis.

In the United States, deep fungal infections are caused by *Histoplasma capsulatum* (endemic eastern Great Lakes, Mississippi, and Missouri river beds); *Coccidioides immitis* (endemic southwestern United States, northern Mexico), or *Blastomyces dermatitidis* (endemic Great Lakes, Saint Lawrence, Ohio, and Mississippi river beds, southeastern united States). The disease states, modes of recognition, and other salient properties of each of these pathogens are described.

Opportunistic fungi include *Aspergillus fumigatus, Candida albicans, Cryptococcus neoformans, Mucor* species, *Rhizopus* species, *Absidia* species, and *Pneumocystis carinii.* The compromising condition leading to infection, the diseases caused, the modes of identification, and other significant features describing each of these organisms are described.

Review Questions

1. An obese 32-year-old diabetic woman presents with complaint of red and painful skin in her abdominal skin folds. Examination reveals a creamy white material at the base of the fold. It is erythematous underneath and extends beyond the creamy material. Microscopic examination of the exudate reveals oval budding structures (3 × 6 μm) mixed with more budding elongated forms. The most likely causative agent is

   A. *Aspergillus fumigatus*
   B. *Candida albicans*
   C. *Epidermophyton floccosum*
   D. *Microsporum canis*
   E. *Sporothrix schenckii*

2. What fungus causes tinea capitis or ringworm infection of the scalp?

   A. *Aspergillus fumigatus*
   B. *Microsporum canis*
   C. *Epidermophyton floccosum*
   D. *Candida albicans*
   E. *Sporothrix schenckii*

3. An 18-year-old high school student in rural north Mississippi develops fever, cough, and chest pain. The cough, associated with weight loss, persisted. Because of poor performance at football practice he was advised to see a physician. Lymph node biopsies stained with H and E studies revealed granulomatous inflammation and macrophages engorged with oval structures measuring 2–4 μm. Cultures incubated at room temperature grew powdery white colonies, which on microscopic study had tuberculate spores. The high school student most likely acquired the infection from

   A. Desert sand
   B. Cat feces
   C. Soil enriched with bird excrement
   D. Another human via respiratory secretions
   E. Contaminated drinking water

4. The most common portal of entry in *Blastomyces dermatitidis* infection is

   A. Mouth
   B. Circulatory system
   C. Skin
   D. Respiratory tract
   E. Central nervous system

5. For which pathogen is infection triggered by traumatic contact with plants on which the organism is growing?

   A. *Candida albicans*
   B. *Coccidioides immitis*
   C. *Cryptococcus neoformans*
   D. *Histoplasma capsulatum*
   E. *Sporothrix schenckii*

6. Hematoxylin and eosin stain of a biopsy specimen from an AIDS patient shows spherules with endospores. The most likely organism is

   A. *Blastomyces dermatitidis*
   B. *Candida albicans*
   C. *Coccidioides immitis*
   D. *Cryptococcus neoformans*
   E. *Histoplasma capsulatum*
   F. *Sporothrix schenckii*

Answers

1. **Answer: B.** Cutaneous candidiasis is a problem in skin folds of obese individuals. It is an even greater problem in diabetic patients because of the high sugar levels. Only the members of the genus *Candida* would produce a creamy surface growth. The erythematous base is due to the production of a cytotoxin. *Aspergillus, Epidermophyton,* and *Microsporum* are all monomorphic filamentous fungi and would not fit the description. *Sporothrix* is found as cigar-shaped budding yeasts but would not clinically present like this. It is traumatically implanted to start subcutaneous infections.

2. **Answer: B.** Tinea capitis or fungal infection of the scalp hair is caused by two genera of the dermatophytes: *Microsporum* and *Trichophyton. Epidermophyton* causes infections of the skin and nails but not the hair. *Microsporum* (the correct answer here) infects hair and nails. The other three fungi do not cause hair infection.

3. **Answer: C.** The clues here are the geography, weight loss, granulomatous inflammation, and macrophages engorged with oval structures (RES disease). The colonial appearance and tuberculate spores strongly suggests the causative agent to be *Histoplasma capsulatum. Histoplasma* is acquired from dusty environments containing bird (most often chicken or starling) or bat feces. The areas of highest endemicity are in the great central river beds with bat caves, chicken coops, and starling roosts having extremely high levels.

4. **Answer: D.** Like most of the systemic fungal agents, this fungus is transmitted by respiratory route. Direct inoculation via skin is possible but not the most common route, even though the species name of this fungus suggests skin.

5. **Answer: E.** Transmission of *Sporothrix* is by break or cut of the skin, whereby the fungus in the contaminated plants or wood is introduced.

6. **Answer: C.** *C. immitis* is the only fungus with endosporulating spherules, demonstrated by either KOH prep or histopathologic stain.

Medical Parasitology

4

What the USMLE Requires You to Know

The USMLE generally does not have many parasitology questions but you will be expected to know the following.

- Name of organism (scientific and common) and major parasite type (e.g., nematode or flagellate).
- Name of disease (common names are frequently used).
- Route of spread, including vector names and reservoir hosts.

For the following organisms you should also know symptoms and understand the pathogenicity:

Entamoeba
Giardia
Plasmodium
Toxoplasma
Cryptosporidium
Enterobius
Ascaris
Hookworms: *Necator* and *Ancylostoma*
Trichinella
Schistosoma

- Review additional bolded material in the following tables.

CLASSIFICATION OF PARASITES

Medical Parasitology is the study of the invertebrate animals and the diseases they cause. Parasites are classified as protozoans or metazoans. The most important organisms in the U.S.A. are identified in the following two tables in boldface type.

Table I-4-1. Protozoans

Common Name	Amebae	Flagellates	Ciliates	Apicomplexa
Important Genera	***Entamoeba*** *Naegleria* *Acanthamoeba*	LUMINAL (GUT, UG) ***Trichomonas*** ***Giardia*** HEMOFLAGELLATES *Leishmania* *Trypanosoma*	*Balantidium*	BLOOD/TISSUE ***Plasmodium*** ***Toxoplasma*** *Babesia* INTESTINAL ***Cryptosporidium*** *Isospora*

Pneumocystis, which was formerly classified as a protozoan, has been determined to be a fungus through ribotyping and other molecular biologic techniques.

Table I-4-2. Metazoans: Worms*

Phylum	Flat worms (Platyhelminthes)		Roundworms
Classes: Common name:	**Trematodes** (flukes)	**Cestodes** (tapeworms)	**Nematodes** ** (roundworms)
Genera:	*Fasciola* *Fasciolopsis* *Paragonimus* *Opisthorchis* (*Clonorchis*) *Schistosoma*	*Diphyllobothrium* *Hymenolepis* *Taenia* *Echinococcus*	***Necator*** ***Enterobius*** *Wuchereria/Brugia* ***Ascaris*** and ***Ancylostoma*** *Toxocara, Trichuris & Trichinella* *Onchocerca* *Dracunculus* Eye worm (*Loa loa*) *Strongyloides*

* Metazoans also include the Arthropoda, which serve mainly as intermediate hosts (the crustaceans) or as vectors of disease (the Arachnida and Insecta).
**Nematodes mnemonic.

HOSTS

The infected host is classified as

- **Intermediate**—host in which **larval or asexual stages develop.**
- **Definitive** —host in which the **adult or sexual stages occur.**

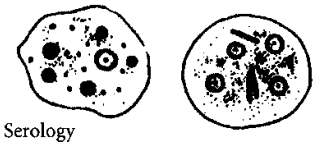
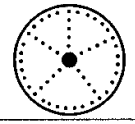
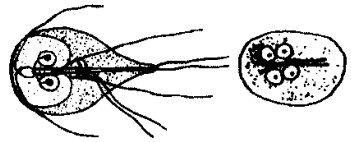
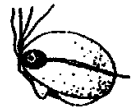
VECTORS

Vectors are **living transmitters** (e.g., a fly) of disease and may be

- **Mechanical,** which transport the parasite but there is no development of the parasite in the vector.
- **Biologic**, in which some stages of the life cycle occur.

IMPORTANT PROTOZOAN PARASITES

Table I-4-3. Protozoan Parasites

Species	Disease /Organs Most Affected	Form/Transmission	Diagnosis	Treatment
Entamoeba histolytica	***Amebiasis***: dysentery **Inverted flask-shaped lesions** in large intestine with extension to peritoneum and liver, lungs, brain, and heart. Blood and pus in stools. Liver abscesses.	Cysts Fecal-oral transmission—water, fresh fruits, and vegetables	Trophozoites: or cysts in stool:  Serology Nuclei have sharp central karyosome and fine chromatin "spokes". 	Metronidazole followed by iodoquinol
Giardia lamblia	***Giardiasis:*** Ventral sucking disk attaches to lining of duodenal wall, causing a fatty, foul-smelling diarrhea (diarrhea → ***malabsorption*** duodenum, jejunum)	Cysts Fecal (human, beaver, muskrat, etc.), oral transmission—water, food, day care, oral-anal sex	Trophozoites or cysts in stool or fecal antigen test (replaces "string" test)  "Falling leaf" motility	Metronidazole
***Cryptosporidium* sp.**	Cryptosporidiosis: transient diarrhea in healthy, severe in immunocompromised hosts	Cysts Undercooked meat, water; not killed by chlorination	Acid fast oocysts in stool: Biopsy shows dots (cysts) in intestinal glands 	
Balantidium coli	***Dysentery***: infection of colon with penetration	Cysts Contaminated food or water	Ciliated trophozoites, cysts in feces 	Tetracycline
Trichomonas vaginalis (urogenital)	Trichomoniasis: often asymptomatic or frothy vaginal discharge	Trophozoites **Sexual**	Motile trophozoites in methylene blue wet mount 	Metronidazole

Free Living Amebae

- Occur in polluted water or soil (*Naegleria, Acanthamoeba*)
- Occur in contact lens saline solutions (*Acanthamoeba*): cysts from dust contaminate

Table I-4-4. Free Living Amebae That Occasionally Infect Humans

Species	Disease / Locale	Form / Transmission	Diagnosis	Treatment
Naegleria	**Primary amebic meningoencephalitis** (PAM): severe pre-frontal headache, nausea, high fever, often an altered sense of smell; often fatal.	Free-living amebae picked up while swimming or **diving in very warm fresh water.**	Motile trophozoites in CSF Culture on plates seeded with Gram-negative bacteria. Amebae will leave trails.	Amphotericin B (rarely successful)
Acanthamoeba	**Keratitis;** **Granulomatous amebic encephalitis** (GAE) in immunocompromised patients: insidious onset but progressive to death.	Free living amebae in contaminated **contact lens solution (airborne cysts)** Not certain for GAE: inhalation or contact with contaminated soil or water.	Star-shaped cysts on biopsy; rarely seen in CSF. Culture as above.	Keratitis: topical miconazole and propamidine isothionate GAE: sulfadiazine (rarely successful)

Plasmodium Species

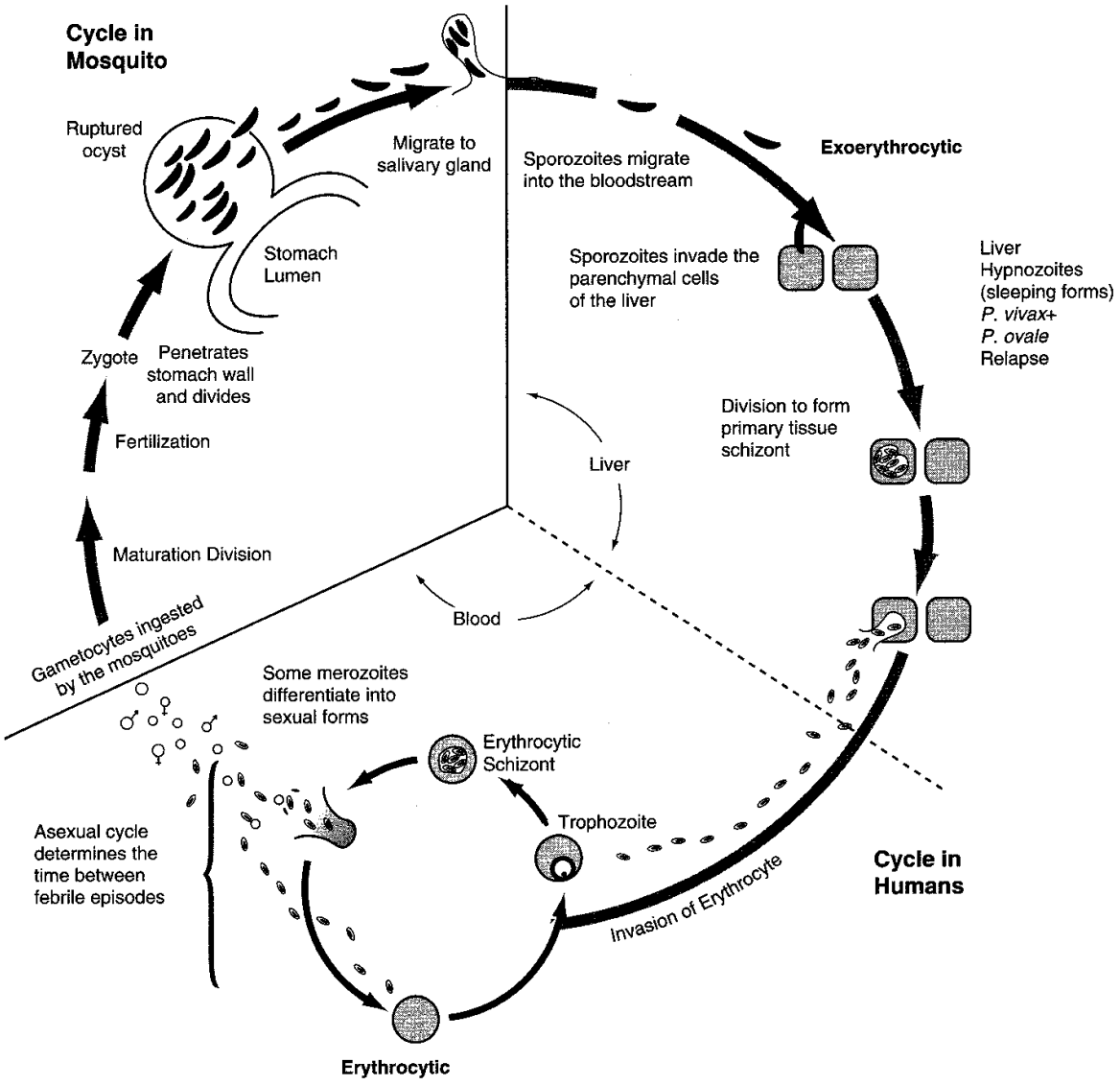


Figure I-4-1. *Plasmodium* Life Cycle

Each *Plasmodium* has two distinct hosts.

- A vertebrate such as the human where asexual phase (schizogony) takes place in the liver and red blood cells.
- An arthropod host (*Anopheles* mosquito) where gametogony (sexual phase) and sporogony take place.

Cause disease by a wide variety of mechanisms, including metabolism of hemoglobin and lysis of infected cells leading to anemia and to agglutination of infected RBC.

Cause paroxysms (chills, fever spike, and malarial rigors) when the infected RBC are lysed, liberating a new crop of merozoites.

Table I-4-5. *Plasmodium* Species

Species	Disease	Important Features	Blood Smears	Liver Stages	Treatment**
Plasmodium vivax	Benign tertian	48-hour fever spikes	Enlarged host cells; ameboid trophozoites	Persistent hypnozoites Relapse*	Chloroquine PO_4 then primaquine
Plasmodium ovale	Benign tertian	48-hour fever spikes	Oval, jagged, infected RBCs	Persistent hypnozoites Relapse	Chloroquine PO_4 then primaquine
Plasmodium malariae	Quartan or malarial	72-hour fever spikes; recrudescence*	Bar and band forms; rosette schizonts	No persistent stage*	Chloroquine PO_4 (no radical cure necessary)
Plasmodium falciparum	Malignant tertian	Irregular fever spikes; causes cerebral malaria	Multiple ring forms crescent-shaped gametes	No persistent stage*	Chloroquine resistance a problem***

*Recrudescence is a reoccurrence of symptoms from low levels of organisms remaining in red cells (recrudescence).
Relapse is an exacerbation from liver stages (hypnozoites).
**Treatment:
1. Suppressive (to avoid infection)
2. Therapeutic (eliminate erythrocytic)
3. Radical cure (eliminate exoerythrocytic)
4. Gametocidal (destruction of gametocytes)
Successful treatment is accomplished with chloroquine followed by primaquine. Chloroquine therapy is suppressive, therapeutic, and gametocidal, whereas primaquine eliminates the exoerythrocytic form.
***Use quinine sulfate plus pyrimethamine-sulfadoxine.

Hemoflagellates (Trypanosomes and Leishmanias)

Hemoflagellates infect blood and tissues.

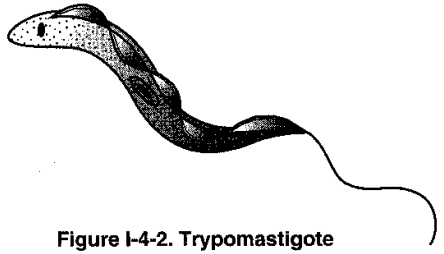


Figure I-4-2. Trypomastigote

Figure I-4-3. Amastigote

Trypanosomes are found

- In human blood as trypomastigotes with flagellum and undulating membrane
- In tissues as **amastigotes (oval cells having neither the flagellum nor undulating membrane)**

Leishmania found always as amastigotes in macrophages.

Table I-4-6. Hemoflagellates

Species	Disease	Vector/Form/Transmission	Reservoirs	Diagnosis	Treatment
Trypanosoma cruzi*	**Chagas' disease** (American trypanosomiasis) Latin America Swelling around eye: (**Romaña's sign**) common early sign Cardiac muscle, liver, brain often involved	**Reduviid bug (kissing or cone bug**; genus *Triatoma*) passes trypomastigote (flagellated form) **in feces** as it bites. Scratching implants in bite site.	Cats, dogs, armadillos, opossums Poverty housing	Blood films	Nifurtimox
Trypanosoma brucei gambiense ***Trypanosoma b. rhodesiense***	**African sleeping sickness** (African trypanosomiasis) **Antigenic variation**	Trypomastigote in saliva of **tsetse fly** contaminates bite	Humans, some wild animals	Blood films, CSF High immunoglobulin levels in CSF	Acute: suramin Chronic: melarsoprol
Leishmania donovani* complex	**Visceral Leishmaniasis** Kalazar	**Sandfly** bite	Urban: humans Rural: rodents and wild animals	Amastigotes in macrophages in bone marrow, liver, spleen	**Stibogluconate sodium** (from CDC)
Leishmania (About 15 different species)	Cutaneous Leishmaniasis (Oriental sore, etc.)	**Sandfly** bite	Urban: humans Rural: rodents and wild animals	Amastigotes in macrophages in cutaneous lesions	Stibogluconate sodium
Leishmania braziliensis complex	Mucocutaneous Leishmaniasis	**Sandfly** bite	Urban: humans Rural: rodents and wild animals	Same	Stibogluconate sodium

**T. cruzi*: An estimated 1/2 million Americans are infected, creating some risk of transfusion transmission in U.S. In babies, acute infections often serious involving CNS. In older children and adults, mild acute infections but may become chronic with the risk of development of cardiomyopathy and heart failure.

***Leishmania* all: Intracellular, sandfly vector, stibogluconate.

Miscellaneous Apicomplexa Infecting Blood or Tissues

Table I-4-7. Miscellaneous Apicomplexa Infecting Blood or Tissues

Species	Disease/Locale of Origin	Transmission	Diagnosis	Treatment
Babesia (primarily a disease of cattle) Humans: *Babesia microti*, WA1, & MO1 strains	Babesiosis (hemolytic, **malaria-like**) Same range as Lyme NE, N Central, California and NW USA	***Ixodes* tick** **Co-infections with *Borrelia***	Giemsa stain of thin smear or hamster inoculation	Clindamycin + quinine
Toxoplasma gondii	See below	**Cat** is **essential definitive** host. Many other animals are intermediate host. Mode: 1) Raw meat in US #1 = pork 2) Contact with cat feces	Serology High IgM or rising IgM acute unfection	Pyrimethamine + sulfadiazine

Toxoplasmosis

Most common parasitic disease.

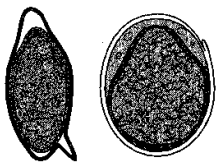
- *Toxoplasma* **acquired after birth is most commonly asymptomatic** or mild, nonspecific with lymphadenopathy and fever. May mimic infectious mononucleosis.
- **Once infected**, as immunity develops, **bradyzoites encyst, but generally remain viable as evidenced by a positive serotiter.**
- Unless prophylactic drugs are given, **AIDS** patients who are seropositive for *Toxoplasma* will have reactivational infections.
- Produces **severe disease in AIDS** or immunocompromised patients.
- **Maternal antibodies protect the fetus,** even if the mother is reinfected during pregnancy.
- If **primary maternal infection** occurs **during pregnancy, the fetus may be infected.**
- **If *Toxoplasma* crosses placenta early, severe** congenital infections: intracerebral calcifications, chorioretinitis, hydro- or microcephaly, convulsions.
- If later, may be inapparent; **untreated inapparent congenital infections lead to progressive blindness.**

IMPORTANT METAZOAN PARASITES

Trematodes

- Are commonly called flukes.
- Are leaf-shaped worms, which are generally flat and fleshy.
- Are hermaphroditic except for *Schistosoma*, which has separate male and female.
- Have complicated life cycles occurring in two or more hosts.
- Have operculated eggs (except for *Schistosoma*), which contaminate water, perpetuating the life cycle, and which are also used to diagnose infections.
- **The first intermediate hosts are snails.**

Table I-4-8. Trematode (Fluke) Diseases

Organism	Common Name	Reservoir Host	Acquisition	Progression in Humans	Important Ova	Treatment
S. mansoni *Schistosoma japonicum*	**Intestinal schistosomiasis**	Cats, dogs, cattle, etc.	Contact with water; skin penetration	Skin penetration (itching) → mature in veins of mesentery → eggs cause granulomas in liver (liver enlargement in chronic cases)		Praziquantel
Schistosoma haematobium	**Vesicular schistosomiasis**	Primates	Contact with water; skin penetration	Skin penetration (itching) → Mature in bladder veins; chronic infection has high association with **bladder carcinoma in Egypt and Africa**		Praziquantel
Non-human schistosomes	**Swimmer's itch**	Birds (Great Lakes U.S.)	Contact with water; skin penetration	Penetrate skin producing **dermatitis** without further development in humans; itching is most intense at 2 to 3 days		Trimeprazine Calamine Sedatives
Clonorchis sinensis	**Chinese liver fluke**	Dogs, cats, humans	Raw fish ingestion		Operculated eggs	Praziquantel
Fasciola hepatica	**Sheep liver fluke**	Sheep, cattle, humans	Ingestion of aquatic plants: water cress		Operculated eggs	Praziquantel
Fasciolopsis buski	**Giant intestinal fluke**	Pigs, dogs, rabbits, humans	Ingestion of aquatic plants: water chestnuts		Operculated eggs	Praziquantel
Paragonimus westermani	**Lung fluke**	Humans, cat family, canines, pigs	Raw crabs, crayfish		Operculated eggs	Praziquantel

Cestodes

- Are the tapeworms.
- Consist of three basic portions: the head or scolex; a "neck" section, which produces the proglottids; and the segments or proglottids, which mature as they move away from the scolex. (The combination of the neck and proglottids is called the strobila.)
- Are hermaphroditic with each proglottid developing both male and female reproductive organs, and mature eggs developing in the most distal proglottids.
- Adhere to the mucosa via the scolex, which is knobby looking and has either suckers or a sucking groove.
- Have no gastrointestinal (GI) tract, they absorb nutrients from the host's GI tract.
- Are diagnosed by finding eggs or proglottids in the feces.
- Have for the most part complex life cycles involving extraintestinal larval forms in intermediate hosts. When humans are the intermediate host, these infections are generally more serious than the intestinal infections with adult tapeworms.

Gastrointestinal Cestodes (Tapeworms)

Table I-4-9. Gastrointestinal Cestodes (Tapeworms)

Cestode (Common name) Intermediate Host (IH) Definitive Host (DH)*	Form/Transmission	Humans Are:	Disease/Organ Involvement/Symptoms (Sx)	Diagnosis	Treatment
Taenia saginata **(beef tapeworm)** IH: cattle DH: humans	Rare beef containing the **cysticerci** is ingested	DH	**Intestinal tapeworm**/Small intestine Sx: Asymptomatic or vague abdominal pains	**Proglottids** or **eggs** in feces	Praziquantel
Taenia solium **(pork tapeworm)** IH: swine; Rarely: humans DH: humans Developing and Slavic countries	Water, vegetation, food contaminated with **eggs** Autoinfection	IH	**Cysticercosis**/eggs → larva develop in brain, eye, heart, lung, etc.	Biopsy	Praziquantel: surgery in some sites
	Rare/raw pork containing the **cysticerci** is ingested by humans	DH	**Intestinal tapeworm** Sx: as for *Taenia saginata*	**Proglottids** or **eggs** in feces	Praziquantel
Diphyllobothrium latum **(fish tapeworm)** IH (2): crustaceans → fish; Rare: humans DH: humans/mammals Cool lake regions	Drinking pond water w/ → copepods (crustaceans) carrying the **larval** forms or frog/snake poultices	IH	**Sparganosis**/larvae penetrate intestinal wall and encyst	Biopsy	Praziquantel
	Rare, raw. pickled fish → containing a **sparganum**	DH	**Intestinal tapeworm** (up to 10 meters)/Small intestine megaloblastic anemia	Proglottids or eggs in feces	Praziquantel
Echinococcus granulosus IH: herbivores; Rare: humans DH: carnivores in sheep-raising areas	Ingestion of eggs	IH	**Hydatid cyst disease** liver & lung where cysts containing brood capsules develop.	Imaging; Serology	Surgery; albendazole
Echinococcus multilocularis IH: rodents DH: canines and cats Northern areas	Ingestion of eggs	IH	**Alveolar hydatid cyst disease**	Tough, as above but no protoscolices	Surgical resection

* Definitive host = adult tapeworm develops in; intermediate host = cysticerci or larvae develop in; cysticerci = encysted larvae found in intermediate host.

Nematodes

- Are the roundworms
- Cause a wide variety of diseases (pinworms, whipworms, hookworms, trichinosis, threadworms, filariasis, etc.)
- Have round unsegmented bodies
- Are transmitted by:
  - ingestion of eggs (*Enterobius, Ascaris,* or *Trichuris*);
  - direct invasion of skin by larval forms (*Necator, Ancylostoma,* or *Strongyloides*);
  - ingestion of meat containing larvae (*Trichinella*); or
  - infection involving insects transmitting the larvae with bites (*Wuchereria, Loa loa, Mansonella, Onchocerca,* and *Dracunculus*).

Table I-4-10. Round Worms (Nematodes) Transmitted by Eggs

Species	Disease/Organs Most Affected	Form/Transmission	Diagnosis	Treatment
Enterobius vermicularis Most frequent helminth parasite in U.S.	**Pinworms,** large intestine, perianal itching	**Eggs**/person to person **autoinfection**	Sticky swab of perianal area Ova have flattened side with larvae inside	Albendazole Treat entire family
Trichuris trichiura	**Whipworm** cecum, appendicitis, and rectal prolapse	**Eggs** ingested	**Barrel-shaped eggs with bipolar plugs** in stools	Albendazole
Ascaris lumbricoides Most common helminth worldwide Largest roundwort	**Ascariasis** Ingest egg → larva migrate thru lungs (cough) and mature in small intestine; may obstruct intestine or bile duct	**Eggs** ingested	**Bile stained, knobby eggs** Adults **6–12"** roundworms	Supportive therapy during pneumonitis; surgery for ectopic migrations; albendazole
Toxocara canis* or *cati (dog/cat Ascarids)	**Visceral Larva Migrans** Larvae wander aimlessly until they die, cause inflammation	**Eggs** ingested/from handling puppies or from eating dirt in yard (pica)	Clinical findings and serology	Albendazole; self-limiting in most cases

Table I-4-11. Roundworms (Nematodes) Transmitted By Larvae

Species	Disease/Organs	Form/Transmission	Diagnosis	Treatment
Necator americanus New World hookworm	**Hookworm** infection Lung migration → pneumonitis bloodsucking → anemia	Filariform **larva penetrates intact skin of bare feet**	Fecal larvae (up to 13 mm) and ova: oval, transparent with 2–8 cell-stage visible inside Occult blood fecal may be present	Mebendazole and iron therapy
Ancylostoma braziliense ***Ancylostoma caninum*** (dog and cat hookworms)	**Cutaneous Larva Migrans**/intense skin itching	Filariform larva penetrates intact skin but cannot mature in humans	Usually a presumptive diagnosis; exposure	Thiabendazole
Strongyloides stercoralis	**Threadworm** strongyloidiasis: Early: pneumonitis, abdominal pain, diarrhea Later: malabsorption, ulcers, bloody stools	Filariform **larva penetrates intact skin;** **Autoinfection** leads to indefinite infections unless treated.	Larvae in stool, serology	Thiabendazole
Trichinella spiralis	Trichinosis: larvae encyst in muscle → pain	**Viable encysted larvae in meat** are consumed: wild game meat.	Muscle biopsy; clinical findings: **fever, myalgia, splinter hemorrhages, eosinophilia**	Steroids for severe symptoms + mebendazole

Wuchereria bancrofti and *Brugia* are filarial worms causing elephantiasis. Both are transmitted by mosquitoes.

Loa loa is the eye worm transmitted by biting flies.

Onchocera volvulus causes river blindness characterized by itchy "leopard" rash and worms in eye. Blackfly transmits.

Dracunculus medinensis is the guinea worm. It is transmitted by drinking infected copepods (cyclops) in water.

Matching

(A) *Ancylostoma braziliensis*
(B) *Ascaris lumbricoides*
(C) *Balantidium coli*
(D) *Diphyllobothrium latum*
(E) *Clonorchis sinensis*
(F) *Cryptosporidium parvum*
(G) *Dermacentor andersoni*
(H) *Echinococcus multilocularis*
(I) *Entamoeba coli*
(J) *Entamoeba histolytica*
(K) *Enterobius vermicularis*
(L) *Giardia lamblia*
(M) *Ixodes scapularis (I. dammini)*
(N) *Leishmania braziliensis*
(O) *Plasmodium vivax*
(P) *Pneumocystis carinii*
(Q) *Pediculus humanus*
(R) *Sarcoptes scabiei*
(S) *Schistosoma haematobium*
(T) *Strongyloides stercoralis*
(U) *Toxoplasma gondii*
(V) *Trichinella spiralis*
(W) *Trichuris trichiura*
(X) *Trichomonas vaginalis*
(Y) *Trypanosoma cruzi*
(Z) *Wuchereria bancrofti*

_____1. Invasive amebae causing dysentery, which is noted for causing extraintestinal abscesses

_____2. Chronic infections associated with bladder carcinoma

_____3. Vector for *Babesia microti*

_____4. Untreatable or at least poorly treatable causative agent of chronic diarrhea in AIDS patients, which is diagnosed by finding acid fast cysts in the stool

_____5. By ribotyping, now considered to be a fungus

_____6. Critically careful surgery is major component of therapy

_____7. Ciliate causative agent of diarrhea

_____8. Filarial worm maturing in the lymphatics and causing elephantiasis

_____9. Adult females live inside adult male groove

_____10. Carrier of epidemic typhus and Trench Fever

_____11. Infection results in enteritis and eosinophilia with flu-like symptoms, periorbital swelling, petechial hemorrhages, and ultimately muscle pain; later in life X-ray may show fine calcifications in the muscle

_____12. Fatty diarrhea associated with malabsorption syndrome

Answers

1. J The organism causing diarrhea but most noted for its ability to leave the gastrointestinal tract is *Entamoeba histolytica*. Remember that *Entamoeba coli* is not a pathogen but rather a commensal organism.
2. S Chronic infections with *Schistosoma haematobium* are notedly associated with bladder carcinoma.
3. M The vector for babesiosis, Lyme disease, and ehrlichiosis is the *Ixodes* tick, *Ixodes scapularis* (*I. dammini*) in the midwest and east, with *Ixodes pacificus* in the western United States.
4. F *Cryptosporidium* treatment is still experimental and not highly effective.
5. P *Pneumocystis carinii* has always stained like a fungus but until ribotyping was considered a protozoan parasite.
6. H Hydatid cyst disease requires delicate surgery to remove them without breaking and releasing the larvae.
7. C *Balantidium* is the only ciliate on the list (and the only one you need to know).
8. Z *Wuchereria* is one causative agent of elephantiasis. *Brugia* is another.
9. S Schistosomes are not hermaphroditic like other flukes (trematodes). They instead have separate sexes, but they live permanently together with the female in a groove of the male.
10. Q The human body louse is a *Pediculus* and carries both epidemic typhus and Trench Fever.
11. V Classical description of trichinosis, causative agent *Trichinella spiralis*.
12. L *Giardia* is probably most noted among the parasites for causing diarrhea with fat malabsorption.

Chapter Summary

Table I-4-1 identifies the important genera in each of the four groups of protozoa, and Table I-4-2 identifies the important genera in each of the three phyla of metazoa.

Table I-4-3 describes the diseases, affected organs, cellular forms, methods of transmission, modes of diagnosis, and treatments for the important pathogenic protozoan species. Table I-4-4 does the same for free-living species that occasionally infect humans.

The life cycle of malaria-causing species is illustrated in Figure I-4-1, and the properties of individual *Plasmodium* species are described in Table I-4-5.

The morphology and properties, as well as the diseases caused by, the vectors responsible for transmission of, the modes of transmission of, the reservoirs for, the diagnoses of, and the treatments for infection by the various species of the *Trypanosoma* and *Leishmania* hemoflagellates are described in Table I-4-6 and Figures I-4-2 and I-4-3.

The most common parasitic disease, toxoplasmosis, is caused by *Toxoplasma gondii* and is transmitted by raw meat or contact with cat feces. It and *Babesia* are described in Table I-4-7.

The trematode organisms, the common names of the diseases they cause, their reservoir hosts, their modes of acquisition, the progression of the diseases they cause in humans, their ova, and treatments for their infections are described in Table I-4-8.

The tapeworms (Cestodes) are described in Table I-4-9; the roundworms (Nematodes) transmitted by eggs are described in Table I-4-10, and the roundworms transmitted by larvae are described in Table I-4-11.

Review Questions

1. B.F., a 44-year-old, returns home to New York following a 2-week camera safari to East Africa. She started chloroquine anti-malarial prophylaxis 2 weeks prior to her departure for Kenya and continued throughout her foreign travel. She stopped taking the pills on her arrival home because they made her nauseated. Two weeks after her return she develops paroxysmal fever and diaphoresis and is quickly hospitalized with febrile convulsions, jaundice, and anemia. Blood smears reveal red blood cells multiply infected with delicate ring-like trophozoites and rare sausage-shaped gametocytes. The stage of the parasite life cycle that is responsible for the appearance of the parasites 2 weeks after departure from the malarious area is the

   A. Hypnozoite
   B. Sporozoite
   C. Exoerythrocytic schizont
   D. Erythrocytic schizont
   E. Merozoite

2. At a school nurse's request, a clinic in rural South Carolina sees a 9-year-old girl who appears listless and inattentive, although hearing and visual testing has been within normal limits. The physician finds the child thin, with the "potbelly" of malnutrition, and orders a fecal exam and CBC. The CBC reveals a microcytic, hypochromic anemia, and the fecal exam detects brown, oval nematode eggs approximately 65 microns in size, too numerous to count. What was the most likely means by which this child was infected?

   A. Ingestion of eggs
   B. Ingestion of larvae
   C. Ingestion of cysts in muscle
   D. Skin penetration by larvae
   E. Mosquito transmission of sporozoites

3. An HIV-positive patient with a CD4+ count of 47 presents with diarrhea. Acid-fast oocysts are found in the stool. From this finding, what is the proper care and prognosis with that care?

   A. Infection is short lasting and self-resolving and requires no treatment
   B. If treated with antibiotics, the infection should resolve in 3–6 days
   C. Infection will resolve only with a combination of anti-tuberculous drugs and then it may take weeks
   D. Infection could have been prevented by avoiding cat feces and undercooked or raw meat
   E. Even with the best treatment, the infection may be unrelenting

4. A 24-year-old primiparous woman in her eighth month of gestation develops a positive IgM titer to *Toxoplasma gondii* for the first time. She should be advised by her physician

   A. That this child and all future fetuses are likely to be infected
   B. That a newborn with a positive anti-Toxoplasma IgG response should be treated with anti-parasitics
   C. That future infections can be avoided by proper vaccination and worming of cats
   D. That retinochoroiditis can be prevented by drug treatment of an infant with a positive IgM response
   E. That major organ damage can be reversed by prompt treatment of the newborn

5. A 35-year-old Captain in the Army Reserves has been plagued by a painful, erosive lesion near his ear lobe since his return from Operation Desert Storm several years ago. He denies exposure to the toxic by-products of burning oil fields. Punch biopsy of the leading edge of the erosion reveals macrophages distended with oval amastigotes. How was this infection acquired?

   A. Contact with contaminated drinking water
   B. Bite of infected Anopheles mosquito
   C. Bite of infected reduviid bug
   D. Fecal contamination of food
   E. Direct human contact in barracks
   F. Bite of sandfly
   G. Bite of tsetse fly

6. A group of six college students undertake to climb Mt. Rainier outside Seattle on their spring break. They pack food and camping provisions except for water, which they obtain from the many fresh water mountain streams that arise at the summit. The adventure takes a little over a week to accomplish, and all return safely and in good spirits to their classes the following week. Within the first week after their return, 5 of the 6 students report to the infirmary with profuse diarrhea and tenesmus. Each affected student experiences weakness and weight loss and stool samples submitted to the lab are yellow, greasy, and foul smelling. What attribute of this parasite imparts its pathogenicity?

   A. Lytic enzymes
   B. Flagella
   C. Ventral sucking disc
   D. Encystment
   E. Toxic metabolites

Answers

1. **Answer: C.** B.F. is suffering from *Plasmodium falciparum* malaria acquired shortly before her departure from Kenya. Liver stages of *Plasmodium* are not susceptible to chloroquine killing. Because she did not continue the prophylaxis after her return to the States, this allowed those parasites to initiate all of the erythrocytic stages of the life cycle. Any erythrocytic stages generated out of the liver phase of the life cycle while she remained on prophylaxis would have been killed. Thus, the late onset of her symptoms was due to survival of exoerythrocytic stages that had not yet left the liver at the time she ceased prophylaxis. Hypnozoites are responsible for relapse of symptoms in *P. vivax* and *P. ovale* malarias, but do not exist in *P. falciparum*, and it is clear that she has *falciparum* malaria due to the delicate ring forms multiply infecting erythrocytes and the sausage-shaped gametocytes. Sporozoites are the infectious forms injected by mosquitoes and would not have been available in this country to initiate the symptoms on the time course described. Erythrocytic schizonts and merozoites would have been killed by prophylaxis before she left Africa and could not be responsible for the late onset of symptoms.

2. **Answer: D.** This child has the typical symptoms of hookworm disease, caused in this country usually by *Necator americanus*. The infection is acquired by penetration of the filariform larvae through the skin of the feet or buttocks, after contamination of soil with the eggs of the agent deposited in human feces. Of the other distractors, choice A would be most likely if the infection were due to ascarids, pinworms, or whipworms. Choice C would describe infection with either *Taenia* or *Trichinella*, and choice E would be the means of infection with *Plasmodium*.

3. **Answer: E.** The described infection is most likely to be *Cryptosporidium*, which is a very difficult infection in AIDS patients even though it is self-resolving in normal noncompromised individuals. In AIDS patients it is most commonly unrelenting, even with treatment. *Cryptosporidium* is usually acquired from water; it is *Toxoplasma* that's from cats.

4. **Answer: D.** The positive IgM titer arising in the eighth month means that this woman has become acutely infected with *Toxoplasma*. Infections acquired at this time have a high likelihood of infecting the fetus and are most likely to be manifested by the development of retinochoroiditis. A mother can transmit this parasite to her fetus only during an acute infection; therefore, all future fetuses will be protected from the disease. Since IgG antibodies cross the placenta, presence of the anti-*Toxoplasma* antibodies of this class in the neonate may simply reflect the infection of the mother—only a positive IgM response in

the neonate is proof of the child's infection, which should therefore be treated. There is no way to reverse major organ damage when it occurs in utero, but it would not be expected to occur with an acute infection beginning in the third trimester.

5. **Answer: F.** *Leishmania* spp. are transmitted by the bite of sandflies. They cannot be transmitted from person to person by trivial means, so unless organ transplantation is occurring in the barracks, direct human contact (**choice E**) is not a possibility. To survive outside the human host, they must be in the vector (sandfly), so transmission by food or water (**choices A** or **D**) are not possible. Of the distractors that involve true vectors: *Anopheles* mosquitoes (**choice B**) transfer malaria; reduviid bugs (**choice C**) transfer American trypanosomiasis (Chagas' disease); and tsetse flies (**choice G**) transmit African trypanosomiasis (sleeping sickness).

6. **Answer: C.** *Giardia* is common in mountain streams throughout the U.S., and the presentation of prolonged fatty diarrhea and weight loss is pathognomonic. It causes its pathology by its adherence to the mucosa of the upper small intestine with its ventral sucking disc. No toxic metabolites or lytic enzymes are involved in the pathology, which apparently results from blockage of normal digestive absorption. The organism is a flagellate, and thus has flagella, but migration into extraintestinal sites is not a well known problem associated with pathology. And although the organism does encyst as it passes along the intestine, this is not known to produce symptoms.

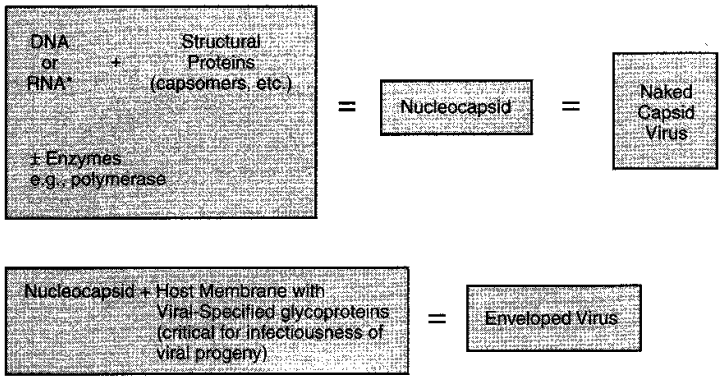
Medically Important Viruses

5

What the USMLE Requires You to Know

- Major concepts of host and tissue specificity
- Major concepts of viral replication
- How viruses cause disease
- Basics of viral diseases (as for bacterial diseases)
- Plus for each virus
  - Nucleic acid (and generalities about how it replicates)
  - Nucleocapsid shape
  - Whether or not it is enveloped

STRUCTURE AND MORPHOLOGY



*Positive sense RNA = (+) RNA
(can be used itself as mRNA)

ss = Single stranded
ds = Double stranded

*Negative sense RNA = (–) RNA
- Complementary to mRNA
- Cannot be used as mRNA
- Requires virion-associated RNA-dependent RNA polymerase (as part of the mature virus)

Figure I-5-1. The Basic Virion

Note

SITV*1 and 2

(Simplified imaginary teaching virus):

- The codon for phenylalanine is UUU.
- Phenylalanine is represented in Figure V-2 by the Greek letter Φ (phi).
- The SIT viruses (SITV + RNA and SITV – RNA) both have a single gene that codes for their capsids, which is made up entirely of phenylalanine.
- Look at the genome in Figure V-2. Which is the positive RNA virus?

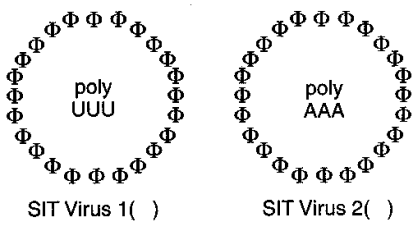


Figure I-5-2. Simplified Imaginary Teaching Viruses

VIRAL STRUCTURE

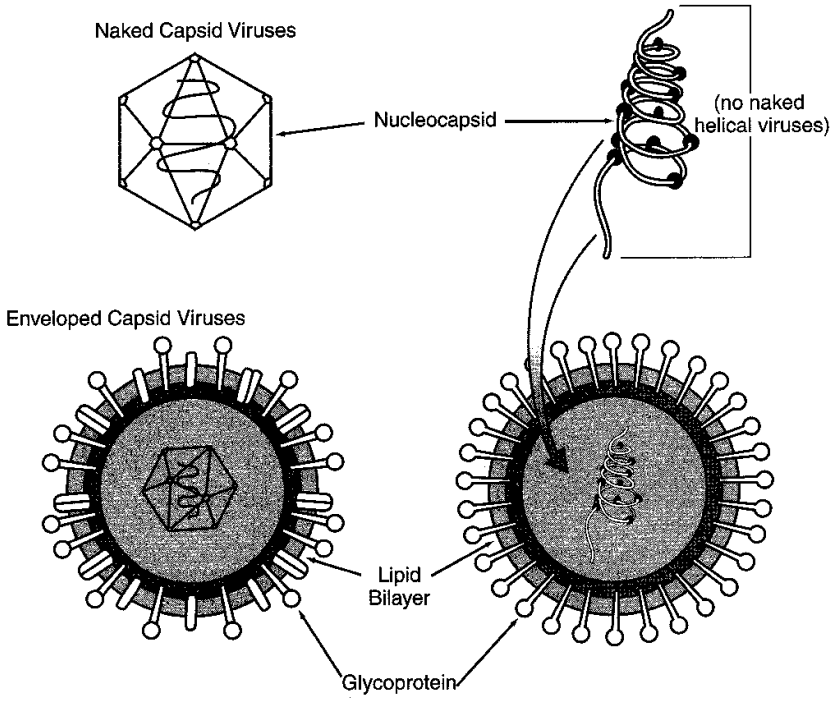


Figure I-5-3. Morphology of Viruses

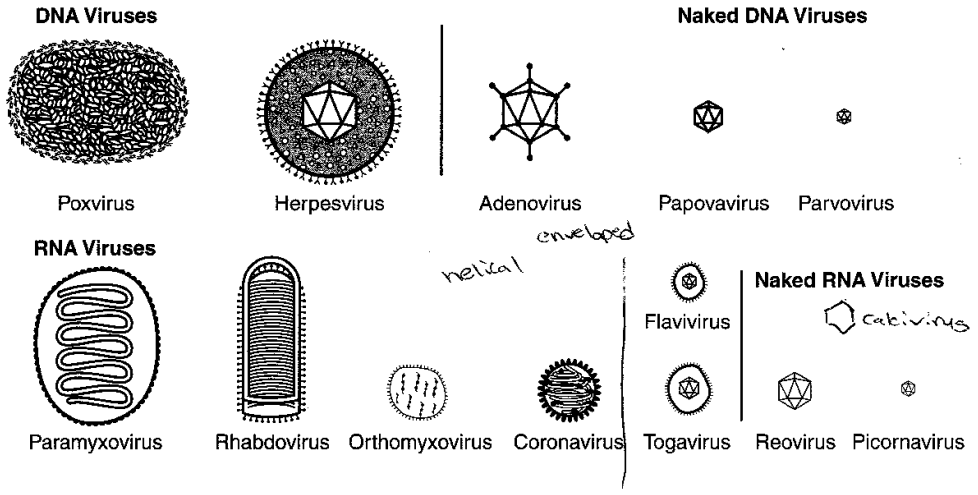
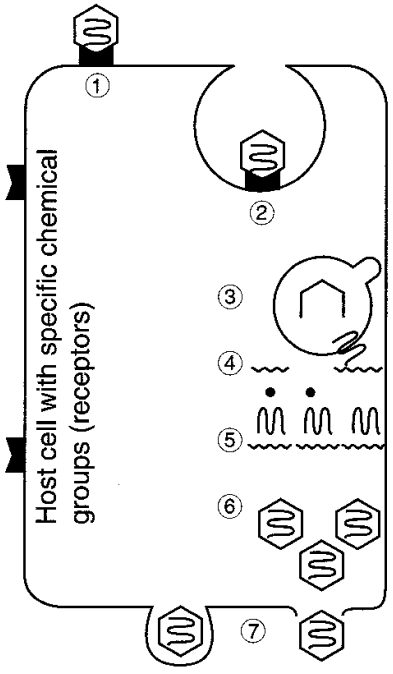


Figure I-5-4. Relative Sizes and Shapes of Different Viruses

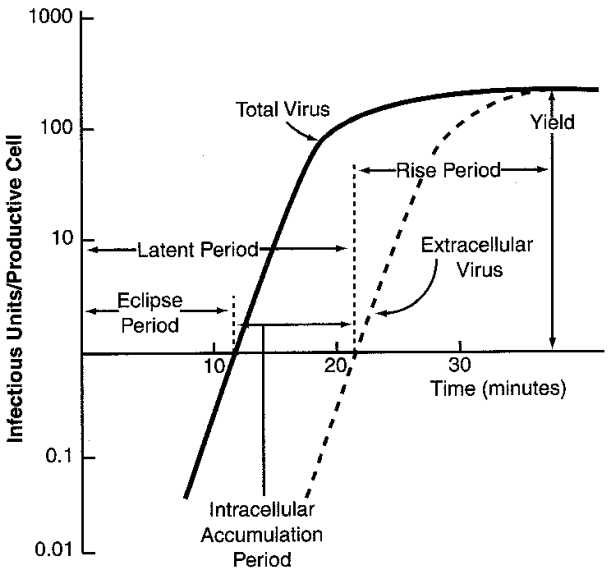
VIRAL REPLICATION



1. **Attachment** to specific host cell receptors: this binding determines what can be infected.
2. **Penetration**
3. **Uncoating** (release of nucleic acid)
4. **Macromolecular synthesis**
   a. **Early** mRNA and protein synthesis:
      Proteins to shut off host cell
      Proteins to replicate viral genome (if needed)
   b. **Replication of the genome**
   c. **Late** mRNA and protein synthesis: structural proteins
5. **Postranslational modification** of proteins
6. **Assembly** of new virus particles
7. **Release** (lysis of the cell or budding out)

Eclipse period: no internal (nor external) virus
Latent period: no external virus

Figure I-5-5. Generalized Viral Replication Scheme



Internal virus is present **after** the end of the eclipse period.
External virus is present **after** the end of the latent period.

Figure I-5-6. One Step Viral Growth Curve

IMPORTANT STEPS IN VIRAL REPLICATION

Spread

Viruses are spread basically by the same mechanisms (e.g., respiratory droplets or sexually) as other pathogens.

Arthropod-borne viruses are referred to as **arboviruses.**

Most belong to three formal taxonomic groups

- Togavirus encephalitis viruses (a.k.a. alphaviruses)
- Flavivirus
- Bunyavirus

Vectors

- Mosquitoes are most common vectors.
- Ticks, biting midges, and sandflies are less common.

Attachment

Viruses bind through specific interaction with the host cell surface components and

- Specific **viral surface glycoproteins** of **enveloped viruses**, or
- Specific **viral surface proteins** of **naked viruses.**

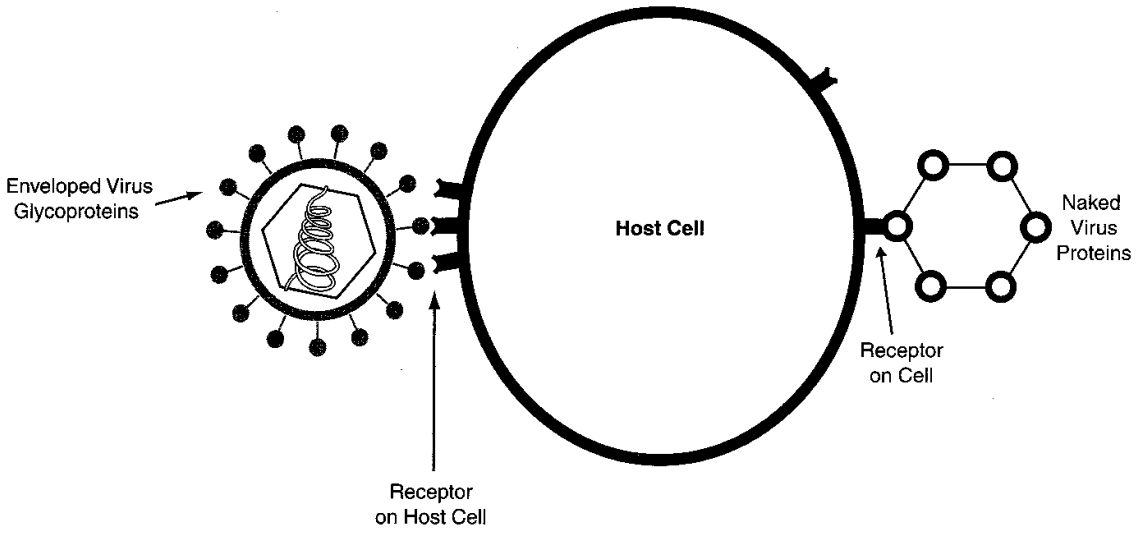


Figure I-5-7. Attachment

These interactions (and the distribution of the receptors) **determine viral**

1. **Host range** (e.g., horses or humans)
2. **Tissue specificity** (e.g., liver versus heart)

Table I-5-1. Specific Viral Receptors to Know

Virus	Target Cell	Receptor on Host Cell
HIV	Th cells, macrophages, microglia	CD4
EBV	B lymphocytes	CD21= CR2
Reovirus	Neurons	β-adrenergic receptor
Rabies	Neurons	Acetylcholine receptor

Table I-5-2. Difference Between Naked and Enveloped Viruses

	Naked	Enveloped
Inactivated by heat, detergents, and organic solvents like ether and alcohols?	No	Yes, since the lipid envelope holds the glycoproteins essential for attachment. Dissolving the envelope inhibits attachment and therefore uptake.
Immune response	Prominently antibody	Antibody and prominent cell-mediated immunity

Viral Entry Into Host Cell

Viral entry is by

- Receptor-mediated endocytosis
- Uptake via coated pits
- Or for those enveloped viruses with fusion proteins via fusion of the cell membrane with the viral envelope

Macromolecular Synthesis

How do the various viruses make their mRNA? mRNA production is diagrammed below.

- The major types of viral genomes are shown on the right.
- The replication intermediates necessary to make mRNA are shown in the gray area.

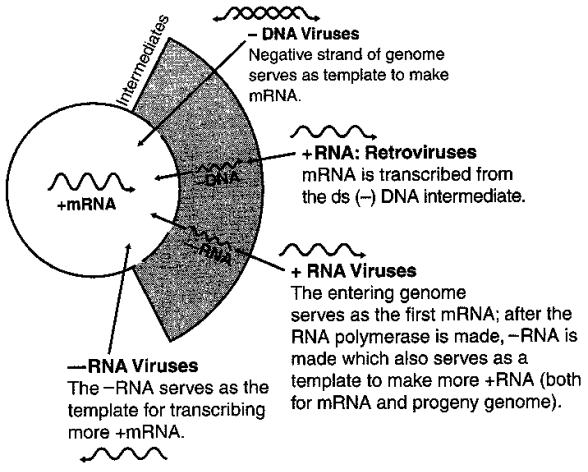

Intermediates
– DNA Viruses
Negative strand of genome serves as template to make mRNA.
+RNA: Retroviruses
mRNA is transcribed from the ds (–) DNA intermediate.
DNA
+mRNA
–RNA
+ RNA Viruses
The entering genome serves as the first mRNA; after the RNA polymerase is made, –RNA is made which also serves as a template to make more +RNA (both for mRNA and progeny genome).
—RNA Viruses
The –RNA serves as the template for transcribing more +mRNA.

Figure I-5-8. Viral Messenger RNA

Replication of the Genomic Nucleic Acid (NA)

Progeny viruses have a nucleic acid sequence identical to the parent virus.

All single-stranded **RNA viruses replicate through a replicative intermediate.**
Going back to the Simplified Imaginary Teaching Viruses:

- If the parental genomic sequence is UUUUUUUUU, then the progeny must have the same sequence.
- (Poly AAA would make a polylysine capsid instead.)
- To make more poly UUU, a replicative intermediate of AAAAAAAAA would be required.
- The replicative intermediate is used to make new poly UUU.

Table I-5-3. Strategy for Viral Genome Replication

Virus Type	Parental Genome	Intermediate Replicative Form	Progeny Genome
Most +ssRNA viruses	+ssRNA	–ssRNA	+ssRNA
Retroviruses	+ssRNA →	dsDNA	+ssRNA
–ssRNA viruses	–ssRNA	+ssRNA	–ssRNA
Most dsDNA viruses	dsDNA		dsDNA
Hepatitis B	dsDNA	ssRNA →	dsDNA

– means an RNA which can serve as mRNA (or for the retroviruses has the same sequence.)

→ = RNA-dependent DNA polymerase

- Called reverse transcriptase for the retroviruses.
- Called the DNA polymerase for hepatitis B.
- Both actually make the first strand of the DNA using the RNA template and then breakdown the RNA and use the single strand of DNA as template to make the second strand.

Table I-5-4. Analogies between Cellular* Nucleic Acid and Viral Nucleic Acid

Cellular DNA Coding Strand Similar/Analogous to:	Cellular DNA Template Strand Similar/Analogous to:
Cellular mRNA (T/U substitution)	No major class of "negative" cellular RNA "Antisense" RNA may occasionally be formed to control gene expression
Positive strand of viral DNA	Negative strand of viral DNA
Positive strand of viral RNA (serves as mRNA for virus)	Negative strand of viral RNA (used as template to produce positive mRNA)

*Cellular DNA includes both eukaryotic and prokaryotic cells.

Release of Viruses

Naked viruses lyse the host cells. Thus, there are **no persistent productive infections** with naked viruses (only cytolytic productive or latent infections).

Release of enveloped viruses: Budding leads to cell senescence (aging), but cells may produce a low level of virus for years as occurs in chronic hepatitis B.

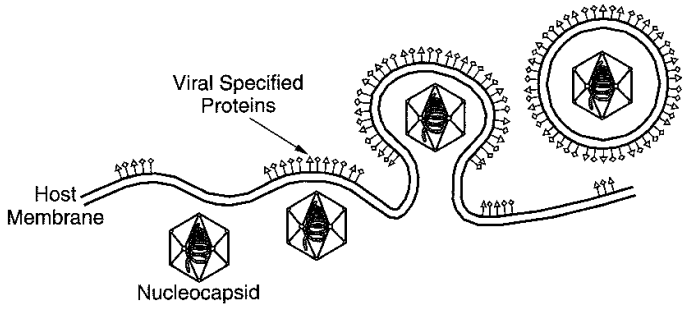


Figure I-5-9. Release of Enveloped Virus

The glycoproteins on the enveloped viral surface are essential for viral infectivity.

PATTERNS OF VIRAL INFECTIONS

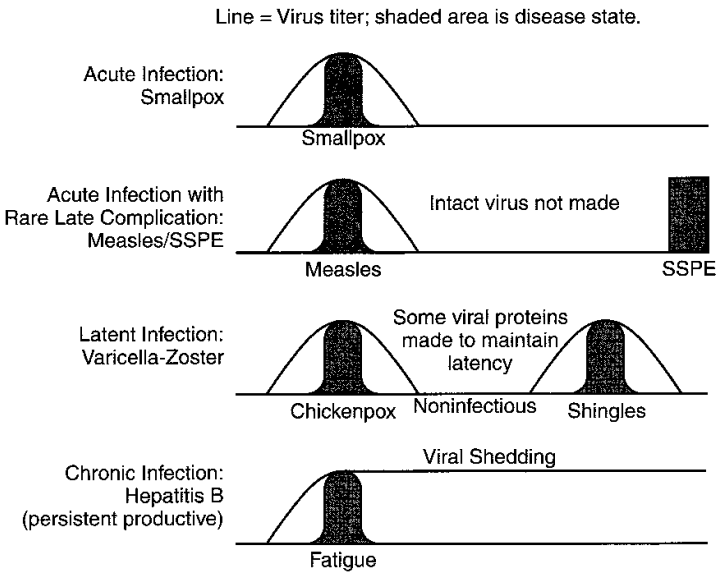


Figure I-5-10. Time Courses: Acute and Persistent Viral Infections

Table I-5-5. Cellular Effects

Infection Type	Virus Production	Fate of Cell
Abortive	–	No effect: No virus is made nor is latency established; Virus is terminated
Cytolytic Naked viruses lyse host cells. Some enveloped viruses also are cytolytic, killing the cell in the process of replication.	+	Lysis of the host cell (death)
Persistent		
Productive (enveloped viruses)	+	Senescence (premature aging)
Latent	–	No overt damage to host; no production of virus, but viral production may be turned on later.
Transforming	±	Immortalization

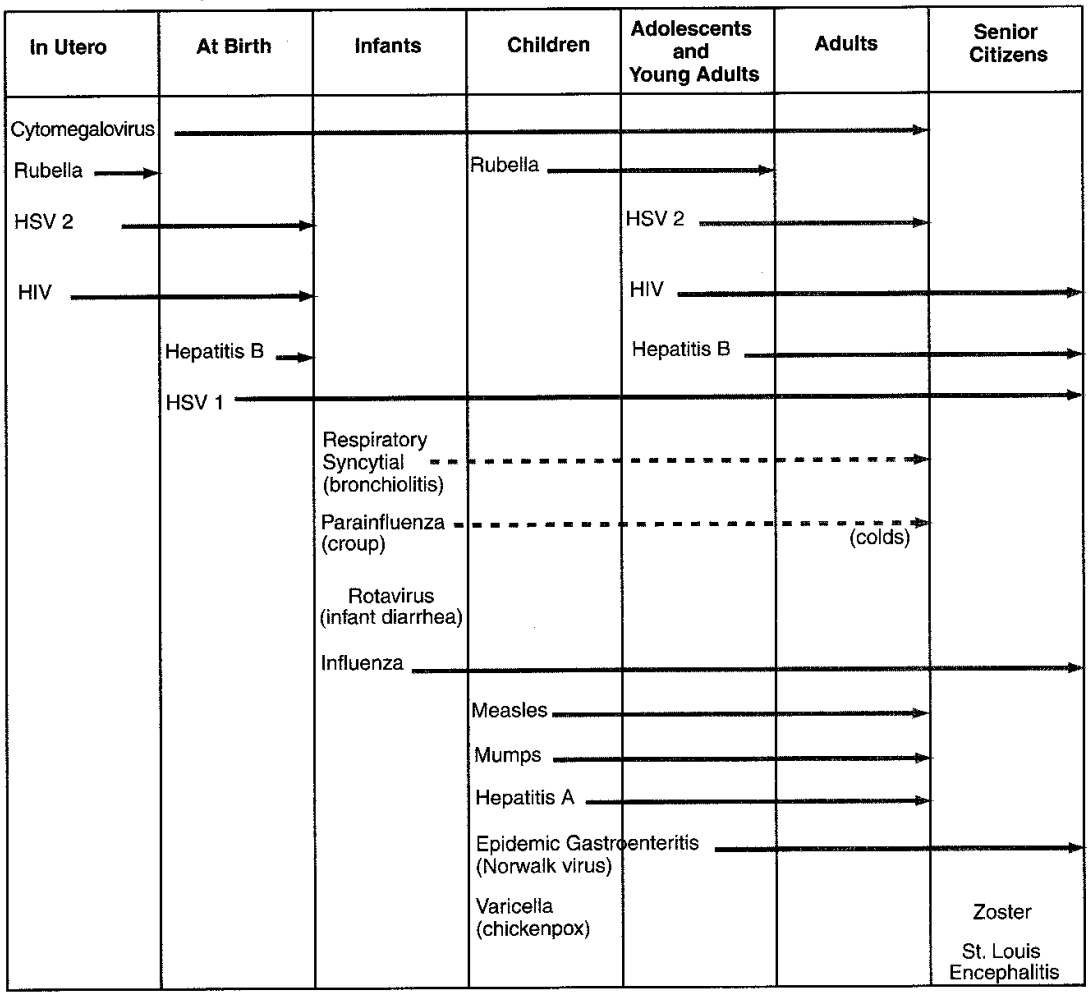


Figure I-5-11. The Most Common Age Groups for Viral Infections

HOST RESISTANCE TO VIRAL INFECTION

Primary Defenses

- Skin barrier (dead keratinized cells impervious to viruses)
- Skin has acids and other inhibitors produced by normal bacterial flora
- Mucociliary elevator

Immune Defense

Innate immune response

- Interferon
- Complement
- Natural killer cells

Adaptive immune response

- Antibody
- Lymphocyte-mediated response

Interferon Production

Interferons (IFNs) are a family of eukaryotic cell proteins classified according to the cell of origin. IFN-alpha and IFN-beta are produced by a variety of virus-infected cells. They:

- Act on target cells to **inhibit viral replication.**
- Do not act directly on the virus.
- Are **not virus-specific.**
- Are **species-specific** (e.g., mouse IFN versus human IFN).

Interferon inhibits viral protein synthesis

- Through activation of an RNA endonuclease, which digests viral RNA.
- By activation (by phosphorylation) of protein kinase that inactivates eIF2 inhibiting viral protein synthesis.

Exogenous human IFN (produced by recombinant DNA technology) may be used in antiviral therapy for chronic, active HBV and HCV infections.

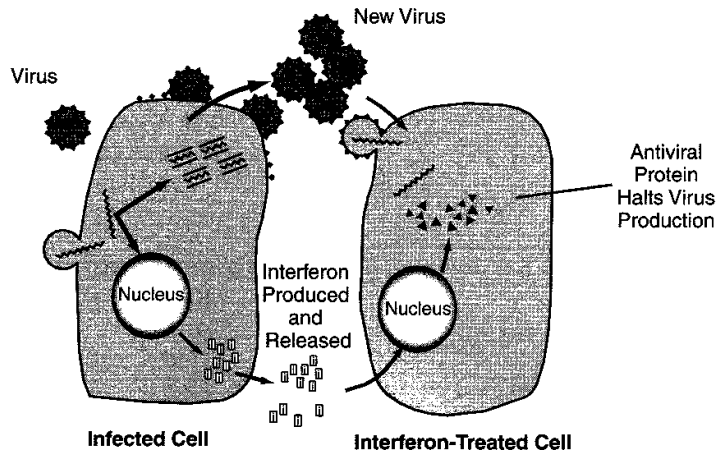


Figure I-5-12. Interferon Production

IMMUNOPROPHYLAXIS

Table I-5-6. Vaccines

	Attenuated (Live)	Killed	Component
Can it revert to a pathogenic form?	Possibly	No	No
Can it cause infections in immunocompromised hosts?	Sometimes	No	No
Immunogenicity?	High	Lower	Middle
Special storage?	Yes; viable organisms	No	No
Potential for contamination with other viruses?	Yes; high	Reduced	No

Active Immunization—Killed Vaccines

RIP-A (Rest In Peace Always—the killed viral vaccines):

Rabies (killed human diploid cell vaccine) and immunoglobulins

Influenza

Polio (Salk)

A Hepatitis

Active Immunization—Live Viral Vaccines

All but adenovirus are attenuated

(mnemonic: Mr. V.Z. Mapsy)

Mumps

Rubella

Varicella - Zoster

Measles

Adenovirus (pathogenic [not attenuated] respiratory strains given in enteric coated capsules)

Polio (Sabin)

Small Pox

Yellow Fever

Active Immunization—Component Vaccines

Hepatitis B

Passive Immunotherapy: Transfer of Immunoglobulins

Hepatitis A
Hepatitis B
Measles
Rabies
Varicella-Zoster

VIRAL HEPATITIS

Symptoms of Hepatitis

Fever, malaise, headache, anorexia, vomiting, dark urine, jaundice.

Table I-5-7. Hepatitis Viruses (Hepatotropic)

Virus	Hepatitis A	Hepatitis B	Hepatitis C	Hepatitis D	Hepatitis E
	"Infectious" (HAV)	"Serum" (HBV)	"Post transfusion Non A Non B" (HCV)	"Delta" (HDV)	"Enteric" (HEV)
Family Features	Picornavirus RNA Naked Capsid	Hepadnavirus DNA Enveloped	Flavivirus RNA Enveloped	Defective Circular RNA Enveloped	Calicivirus RNA Naked capsid
Transmission	Fecal-oral	Parenteral, sexual	Parenteral, sexual	Parenteral, sexual	Fecal-oral
Disease No chronic	Mild acute No chronic No sequellae	Acute is occasionally severe Chronic: 5–10% adults 90% infants 1′ hepatocellular carcinoma, cirrhosis	Acute is usually subclinical 80% become chronic Primary hepatocellular carcinoma, cirrhosis	Co-infection with HBV: occasionally severe; Superinfection with HBV: often severe Cirrhosis, fulminant hepatitis	Normal patients mild Pregnant patients: severe No chronic
Mortality	<0.5%	1–2%	0.5–1%	High to very high	Normal patients 1–2%; pregnant patients: 20%
Diagnosis **acute** hepatitis-symptoms &:	IgM to HAV	HBsAg, IgM to HBcAg	Antibody to HCV ELISA	Hepatitis D Ab, HBsAg	Antibody to HEV ELISA

Remember that hepatitis also may occur in other viral diseases (e.g., CMV and EBV infections, congenital rubella, and yellow fever).

Hepatitis B (HBV) Terminology and Markers

Dane particle = infectious HBV

HBsAg

- Surface antigen
- Found during acute disease and persistent infections.
- Presence of HBsAg past 6 months indicates chronic infection.

HBsAb = Antibody to the surface antigen; provides immunity to HBV.

HBs window is the period **between:**

1. **the end of** detection of **surface antigen and**
2. **the beginning of the detection of surface antibody.**

The absence of these two markers is why the diagnostic test for core antibody is so important.

HBcAg = HBV core antigen

HBcAb = antibody to HbcAg:

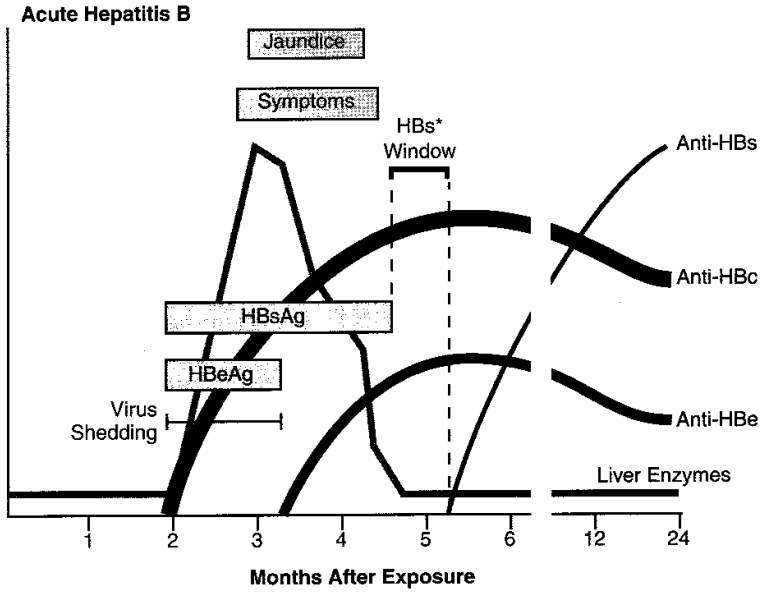
- First antibody to appear
- IgM antibody to core is important test in screening for recent infection.

HBeAg = a second antigenic determinant found in the core of the virus. Its presence correlates with:

- Active viral production
- Infectivity

HBeAb = antibody to HbeAg:

- Generally is detectable after virus is no longer detectable.
- Used to suggest lower risk of transmission.



*The window is the time between the disappearance of the HB surface antigen and before antibody to the surface antigen is detected.

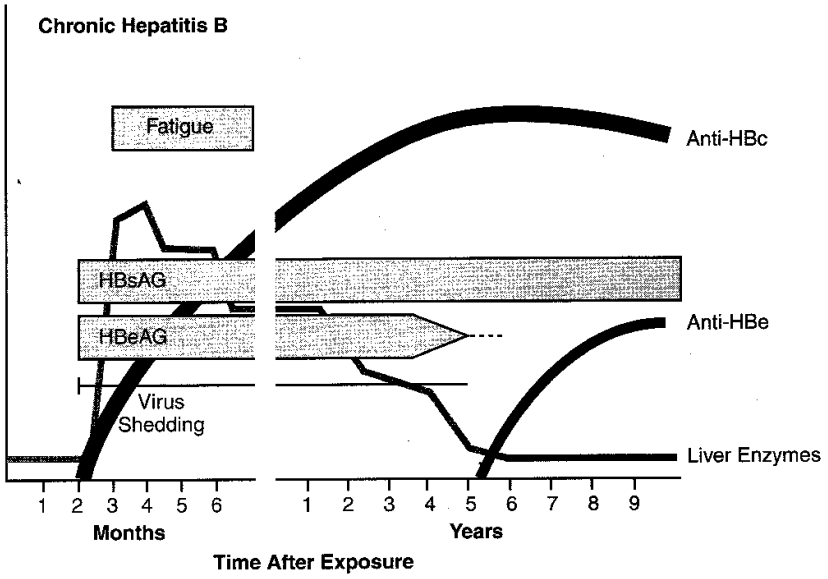


Figure I-5-13. Hepatitis B

DNA VIRUSES: CHARACTERISTICS

All DNA viruses:

- Are double-stranded, except parvovirus
- Are icosahedral, except poxviruses, which are brick-shaped "complex"
- Replicate their DNA in the nucleus, except poxvirus

Table I-5-8. DNA Viruses*

Virus Family	DNA type	Virion-Associated Polymerase	Envelope	DNA Replicates in:	Major Viruses
Parvovirus	ssDNA	No	Naked	Nucleus	B-19
Papovavirus	dsDNA circular	No	Naked	Nucleus	Papilloma Polyoma
Adenovirus	dsDNA linear	No	Naked	Nucleus	Adenoviruses
Herpes virus	dsDNA linear	No	Enveloped (nuclear)	Nucleus; virus assembled in nucleus	HSV Varicella-Zoster Epstein-Barr Cytomegalo-virus
Poxvirus	dsDNA linear	Yes**	Enveloped	Cytoplasm	Variola Vaccinia Molluscum contagiosum
Hepadnavirus	partially dsDNA circular	Yes***	Enveloped	Nucleus, RNA intermediate	Hepatitis B

*Mnemonic: Parva's Papa Adds Her Poxes to Hepa's

** **Poxviruses** have a **virion-associated transcriptase** (DNA dependent RNA polymerase) so it can transcribe its own DNA in the cytoplasm and make all of the enzymes and factors necessary for replication of the poxvirus DNA in the cytoplasm.

*** Hepadnaviruses: DNA viruses that carry a DNA polymerase with reverse transcriptase activity to synthesize an RNA intermediate that is then used to make the genomic DNA. Hepatitis B is partially double-stranded with one complete strand.

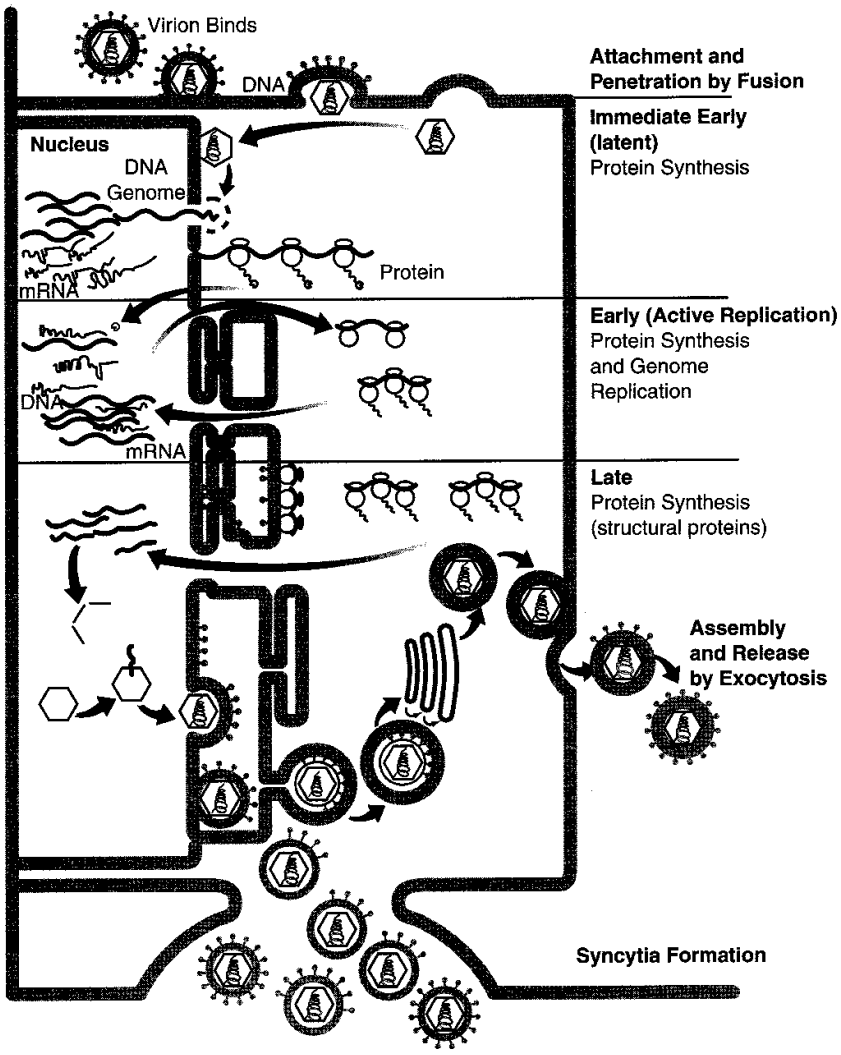


Figure I-5-14. DNA Virus: Life Cycle of Herpes

DNA VIRAL DISEASES

Parvoviridae

Figure I-5-15. Parvovirus

- ssDNA (linear)
- Naked icosahedral

B-19 virus

- Fifth disease = erythema infectiosum = slapped cheek fever:
  A mild, febrile disease with facial rash followed by lacy body rash
- Linked to aplastic crises in Sickle Cell Anemia patients.
  B-19 infects only immature red cells causing lysis, hence the anemia, which is only clinically significant in Sickle Cell Disease.
- Potential cause of hydrops fetalis

Papovaviridae

Figure I-5-16. Papovavirus

- Circular dsDNA
- Naked icosahedral

Human papilloma virus

- Contact
- Plantar warts: HPV 1 & 4
- Anogenital (condyloma acuminatum) and laryngeal papillomas; HPV types 6 and 11 are most common (considered benign).
- Cervical intraepithelial neoplasia (HPV 98%). Types HPV 16 and 18 are the most common causes of neoplasia.
  U.S.A. deaths 4,000–5,000/year
- Pathological findings: koilocytic cells on Pap smear
  (perinuclear cytoplasmic vacuolization and nuclear enlargement)
- Some strains of HPV associated with cancer; these have two genes which inactivate tumor suppressor genes.

Polyoma viruses

- BK virus: in immunocompromised hosts, causes renal disease
- JC virus: causes progressive multifocal leukoencephalopathy
  (slow conventional virus).

Adenoviruses

- dsDNA, naked
- Hexons, penton bases, and fibers
- Fibers bind host cell receptors (will bind to most human cells and many animal cells) and also act as hemagglutinins. Fibers, when purified, are toxic to human cells.

Adenovirus Diseases Include:

- Upper respiratory disease in kids.
- Pharyngoconjunctivitis ("pink eye" or "swimming pool" conjunctivitis), non-purulent (unlike *Haemophilus aegyptius*).

- Epidemic keratoconjunctivitis ("shipyard" conjunctivitis: patients having foreign particles removed acquired these viruses from equipment used on the eyes).
- Acute respiratory disease (ARD) and pneumonia:

  Major problem in young military recruits

  Serotypes 4, 7, and 21.

  Three separate vaccines are used by the U.S. military
  - live non-attenuated
  - serotypes 4, 7, and 21
  - administered separately by enteric-coated capsules
- Adenovirus 40 and 41 cause infantile diarrhea.
- Adenovirus serotypes 12, 18, and 31 are common in *normal* human feces and are not known to cause human disease. These same strains cause malignant transformation of hamster cells. The adenoviruses are the standard examples of a **permissive host (where virus is produced)** and **nonpermissive host (where the virus is not produced but transforms the cells).**

Herpesviridae

Enveloped icosahedral nucleocapsids with dsDNA

DNA is synthesized in the nucleus.

Can enter the **latent state** in the host:

- HSV in neurons
- EBV in B lymphocytes

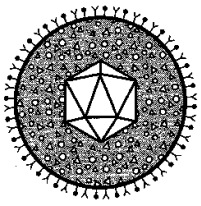


Figure I-5-17. Herpesvirus

Herpes viruses synthesize their own enzymes to synthesize DNA.

Acyclovir (the prodrug form of a nucleoside analog) is effective only in Herpes virus–infected cells because the herpes thymidine kinase is required to activate it, then it inhibits only the herpes polymerase, leaving the host polymerase functioning. No current drug can remove the latent DNA.

Herpes **envelope is from the host nuclear membrane** (virus is assembled in the nucleus).

Herpes form **intranuclear inclusion bodies.**

Herpes produce **distinctive cytopathology.**

Herpes simplex type 1 (HSV-1)

- Gingivostomatitis and recurrent cold sores (skin or lip); **latent in trigeminal root ganglion**
- Keratoconjunctivitis generally with lid swelling and vesicles; dendritic ulcers may be seen; untreated repeated attacks may result in visual impairment.
- Meningoencephalitis
  - Characterized by: fever, headache, and confusion (focal temporal lesions; perivascular cuffing)
  - Diagnosis/treatment STAT: PCR on CSF; **RBCs in CSF**; acyclovir: early diagnosis reduces mortality

Herpes simplex type 2 (HSV-2)

- Meningitis: mild self-limiting disease
- Herpes genital infections: painful vesicular lesions of genitals and anal area; **latent in sacral nerve ganglia**
- Neonatal herpes may be one of three presentations:
  1. Disseminated with liver involvement; high mortality;
  2. Encephalitis; high mortality;
  3. Skin, eyes, or mouth.
- Diagnosis:

  PCR (CSF for encephalitis)

  Viral culture with fluorescent antibody stain to identify virus
- The **Tzanck smear** (Giemsa stain to show multinucleated giant cells) has **been largely replaced by immunofluorescent staining, which can distinguish HSV-1 from HSV-2.**

Varicella-zoster

- Chickenpox
  - Asynchronous rash
  - **Latent in dorsal root ganglia** → shingles in adults (severe nerve pain)
- Associated with Reye's syndrome
- Disseminated infections in immunocompromised hosts
- Attenuated vaccine
- Passive transfer of immunity with Varicella-Zoster immunoglobulin

Epstein-Barr virus (EBV)

- Selectively **infects B cells binding to CD21 = CR2**
- Many inapparent infections; common, worldwide 90% of the population seropositive
- Infectious mononucleosis, "Kissing disease"
  - **Heterophile positive mononucleosis**, fatigue, fever, sore throat, lymphadenopathy, and splenomegaly
  - Atypical reactive T lymphocytes (Downey Type II cells) may become as high as 70% WBC.
  - Positive for heterophile antibodies that cross-react with Paul-Bunnell antigen on sheep and bovine RBC (only mono that is heterophile antibody positive).
  - Antigens produced by productive cells:

    EA = early antigen

    VCA = viral capsid antigen used in diagnostic tests

    EBNA = Epstein-Barr nuclear antigen

    MA = membrane antigen
- Burkitt lymphoma, nasopharyngeal cancer, thymus carcinoma: EBNA found in all transformed B lymphoid cells.

Cytomegalovirus (CMV)

- **Herpesviridae infecting fibroblasts**; common 80% worldwide.
- **Owl's eyes** = CMV "Sightomegalovirus" basophilic intranuclear inclusion bodies with smaller eosinophilic cytoplasmic inclusion bodies.

Table I-5-9. Acquisition Routes for CMV

Transmission	Patient	Resulting Disease
In utero (**most common in utero infection**)	Fetuses	Ranges from infected but no obvious defects to severe cytomegalic inclusion disease (jaundice, hepatosplenomegaly, thrombocytic purpura, pneumonitis, and CNS damage [periventricular calcifications] to death)
Birth process, milk	Babies	Serious disease uncommon, heterophile negative mononucleosis
Sex, transfusions	Healthy adults	Serious disease uncommon, heterophile negative mononucleosis
Reactivation in transplanted organ	Transplant patients	Interstitial pneumonitis and systemic disease
Reactivation or new acquisition	AIDS patients	CMV retinitis, pneumonitis, and systemic disease

Human herpesvirus 6

Exanthem subitum (Roseola): common infant disease; fever followed by rash.

Human herpesvirus 8

Probable **cofactor in Kaposi sarcoma**

Poxviridae

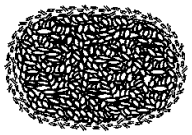


Figure I-5-18. Poxvirus

Complex morphology: Brick-shaped large viruses with ds linear DNA

Virus-coded lipid on surface

Virion-associated DNA-dependent RNA polymerase allows replication in cytoplasm.

Variola

- Smallpox
- Extinct in 1977
- Live attenuated Vaccinia vaccine
- Guarnieri bodies found in infected cells (intracytoplasmic)

Vaccinia

Immunogen in smallpox vaccine

Molluscum contagiosum

- Small pink benign wart-like tumors
- Molluscum bodies in central caseous material: oval, **eosinophilic cytoplasmic inclusion bodies**
- Problem in some immunocompromised hosts

Hepadnaviridae

Hepatitis B virus: see earlier notes.

RNA VIRUSES

Generalizations

All RNA viruses are single stranded (ss) except reovirus.

ss (–) RNA viruses carry an RNA-dependent RNA polymerase.

A virion-associated polymerase is also carried by

- Reovirus
- Arenavirus
- Retrovirus (reverse transcriptase)

Most are enveloped; only **naked** ones are

- Picornavirus
- Calicivirus
- Reovirus

Some are **segmented** (different genes on different pieces of RNA)

- Reovirus
- Orthomyxovirus
- Bunyavirus
- Arenavirus
  (ROBA sounds like robot, pieces)

Table I-5-10. Positive-Sense RNA Viruses*

Virus Family	RNA Structure	Virion-Associated Polymerase	Envelope	Shape	Multiplies in	Major Viruses
Calicivirus	ss(+)RNA Linear Non-segmented	No polymerase	Naked	Icosahedral	Cytoplasm	Norwalk agent Hepatitis E
Picornavirus	ss(+)RNA Linear Non-segmented	No polymerase	Naked	Icosahedral	Cytoplasm	Polio** ECHO Enteroviruses Rhino Coxsackie Hepatitis A
Flavivirus	ss(+)RNA Linear Non-segmented	No polymerase	Enveloped	Icosahedral	Cytoplasm	Yellow fever Dengue St. Louis encephalitis Hepatitis C
Togavirus	ss(+)RNA Linear Non-segmented	No polymerase	Enveloped	Icosahedral	Cytoplasm	Rubella WEE, EEE Venezuelan encephalitis
Coronavirus	ss(+)RNA Linear Non-segmented	No polymerase	Enveloped	Helical	Cytoplasm	Corona-viruses
Retrovirus-	Diploid ss(+)RNA Linear Non-segmented	RNA dep. DNA polymerase	Enveloped	Icosahedral or truncated conical	Nucleus	HIV HTLV Sarcoma

*Mnemonic: (+) RNA Viruses: Call Pico and Flo To Come Rightaway

**Mnemonic: Picornaviruses PEE Co Rn A Viruses
Polio, Entero, Echo, Coxsackie, Rhino, Hep A

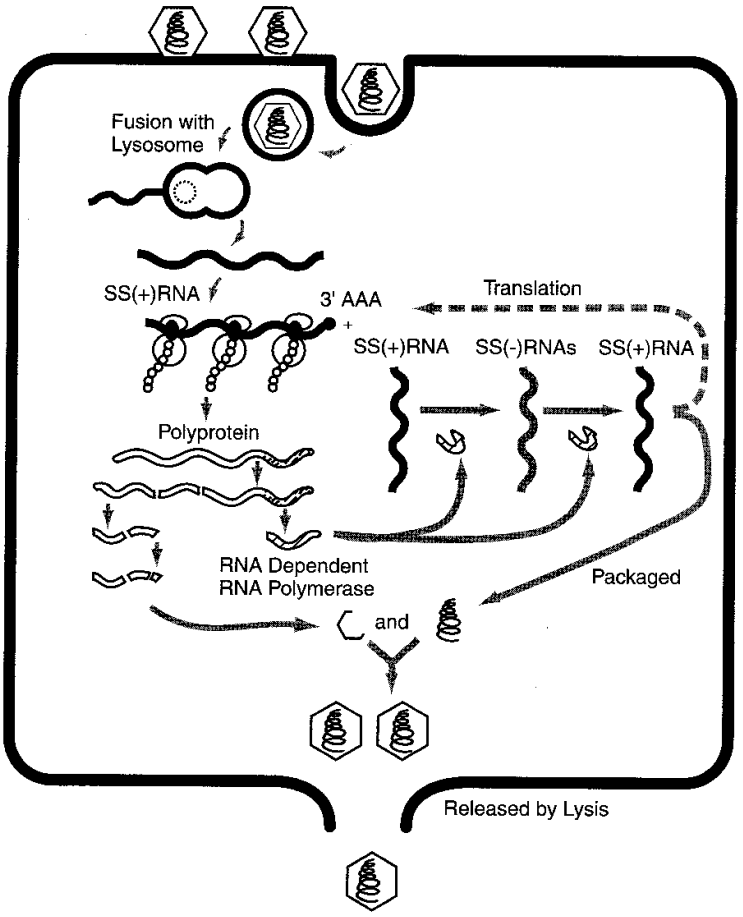


Figure I-5-19. Positive Sense RNA Virus Life Cycle

Abbreviations:

H = Hemagglutinin—surface glycoproteins that bind to sialic acid (N-acetylneuraminic acid) receptors

N = Neuraminidase—clips off sialic acids, thus aiding in release of virus

M = Matrix protein—membrane stabilizing protein underlying the viral envelope

F = Fusion protein—destabilizes host membrane

P = Polymerase associated with virion

Caliciviridae

Small (slightly larger than picornaviridae) but similar to picornaviridae

Norwalk agent

- Described as calicivirus-like.
- Naked icosahedral
- Epidemic viral gastroenteritis in school-age kids and adults

Hepatitis E

See hepatitis notes.

Picornaviridae

- Small ss (+) RNA viruses
- Naked

Figure I-5-20. Picornavirus

Enteroviruses (group)

- Summer-fall peak incidence
- Fecal-oral transmission but do not cause diarrhea
- Peak age group <9 years for most
- Stable at pH3
- Resistant to alcohol, detergents because there is no envelope

Polio Virus

- Most infections are asymptomatic; small % cause fever (viremia).
- Smaller % cause aseptic meningitis.
- Poliomyelitis (paralysis) (even smaller %) results from viral damage to **anterior horn motor neurons.**
- **Vaccines:** both are trivalent
  - Sabin (live/oral/best gut immunity)
  - Salk (killed/injectable)

Coxsackie A

- Herpangina (vesicles on soft palate and fauces)
- Hand-foot-and-mouth disease (oral lesions primarily in the anterior buccal mucosa)
- Aseptic meningitis
- Acute lymphoglandular pharyngitis
- Common cold (aachoo's)

Coxsackie B

- Bornholm's disease (a.k.a., pleurodynia or Devil's grip; severe intercostal pain, fever)
- Aseptic meningitis
- Severe systemic illness of newborns
- Possible link to acute-onset, insulin-dependent diabetes in young children
- Myocarditis

Hepatitis A Virus

- ss (+) RNA
- Infectious hepatitis
- Inactivated vaccine
- Hyperimmune serum for post-exposure prophylaxis

Echoviruses and Most Enteroviruses: Aseptic meningitis

Non-Enteroviruses (group)

Rhinoviruses

- The common cold
- Not stable under acidic conditions
- Peaks summer and fall

Flaviviridae

ss (+) RNA icosahedral capsid with envelope

Yellow Fever Virus

- Mosquito-borne (Aedes)
- Liver, kidney, heart, and gastrointestinal mucosa damage
- Attenuated vaccine

St. Louis Encephalitis Virus

- Mosquito-borne (summer)
- Elderly (especially blacks or individuals with hypertension), most likely to have severe disease

Dengue Virus

- Dengue hemorrhagic shock syndrome in previously infected children who are reinfected. Immune enhancement of entry into macrophages.
- Dengue ("break-bone disease"): mild disease with rash and joint or muscle pain
- Mosquito-borne (Aedes)

Hepatitis C

Discussed with hepatitis viruses.

Togaviridae

- ss (+) RNA viruses
- H, no P

Figure I-5-21. Togavirus

Alpha viruses (group)

Equine Encephalitis Viruses: Western, Eastern, and Venezuelan

- All mosquito-borne
- Wild birds are reservoirs.
- Horses are also hosts.

Rubella

- Crosses placenta and is teratogenic.
- Most serious during first 16 weeks gestation
- Congenital rubella = patent ductus arteriosis, pulmonary stenosis, cataracts, microcephaly, deafness
- Attenuated vaccine; single strain—part of MMR

Coronaviridae

- ss (+) RNA, enveloped helical virus
- The large surface glycoprotein spikes give a crown appearance.
- Second most common cause of common cold (peak winter and early spring)

Retroviridae

Diploid ss (+) RNA viruses

Virion-associated reverse transcriptase

Figure I-5-22. Retrovirus

Oncovirus group

Human T-cell Leukemia/Lymphotropic (HTLV)

- Adult T-cell leukemia
- C-type particle (most oncoviruses, centrally located electron dense nucleocapsid)
- Japan, Caribbean, southern U.S.

Lentivirus group

Human Immunodeficiency Virus (HIV); acquired immunodeficiency syndrome (see next section)

HUMAN IMMUNODEFICIENCY VIRUS (HIV)

Structure and Genes of HIV

Positive sense (ss) RNA virus, diploid, non-segmented

Lentivirus in the retrovirus family (not oncogenic)

The HIV virion contains

- Enveloped truncated conical capsid (type D retrovirus)
- Two copies of the ss (+) RNA
- RNA-dependent DNA polymerase (reverse transcriptase)
- Integrase
- Protease

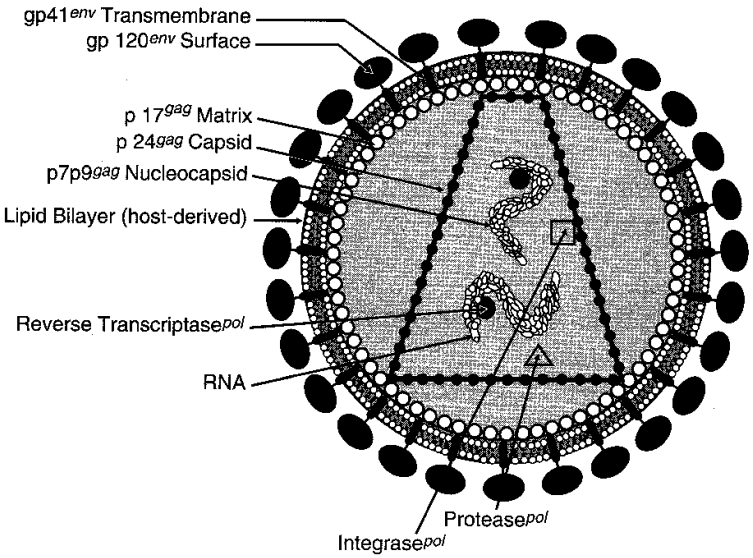


Figure I-5-23. Structure and Genes of HIV

Table I-5-11. Important HIV Genes and Their Functions

Gene	Product(s)	Function
Structural Genes		
Gag	Group specific antigens p24 p7p9 p17	Structural proteins capsid protein core nucleocapsid proteins matrix proteins (stabilizes envelope)
Pol	Reverse transcriptase Integrase Protease	Produces dsDNA provirus Proviral dsDNA integration into host DNA Cleaves polyprotein
Env	gp120 gp41	Surface protein that binds to CD4 on host cell responsible for tropism; genetic drift transmembrane protein for cell fusion
Regulatory Genes		
LTR (U3 U5)	DNA long terminal repeats	Integration and virus gene expression
Tat	Transactivator proteins	Transactivator of transcription; (upregulation); spliced gene
Rev	Regulator virion proteins	Upregulates transport of unspliced and spliced transcripts to the cell cytoplasm; a spliced gene
Nef	Negative factor*	Multiple functions, one of which is to decrease MHCI on infected T cells

*Initially thought to downregulate HIV production; now thought to enhance the pathogenicity of HIV.

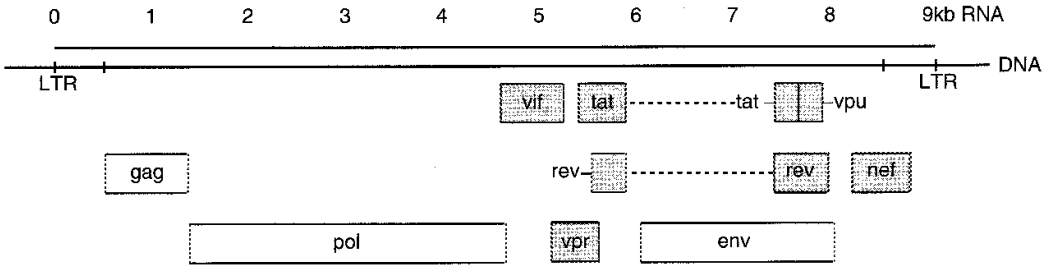


Figure I-5-24. Genetic Map of HIV

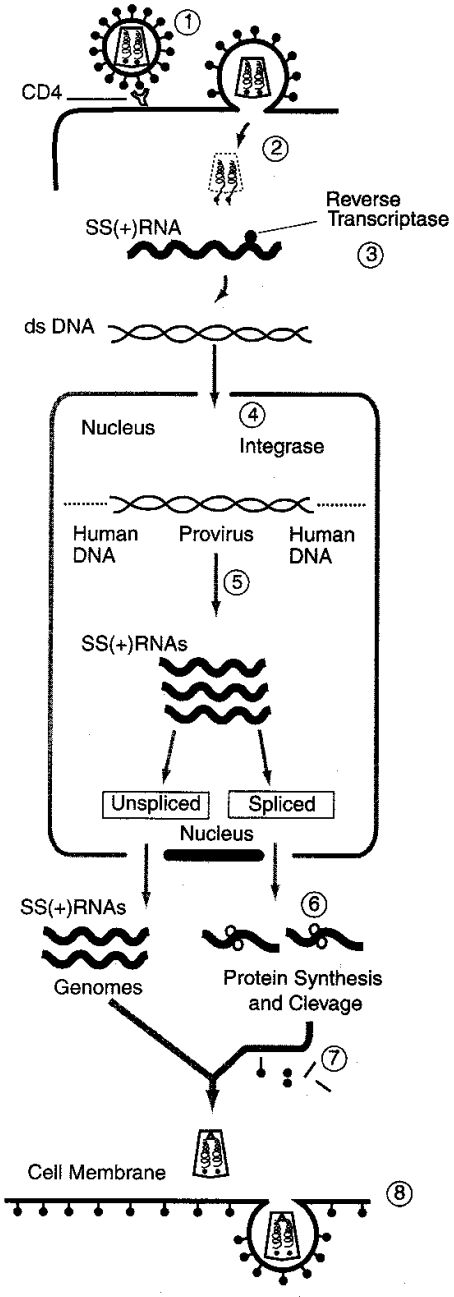


Figure I-5-25. Retrovirus Life Cycle: HIV

1. Surface gp120 of HIV binds to CD4 of T helper cells, macrophages, and microglia.

2. HIV is taken into the cell, losing the envelope; the RNA is uncoated.

3. The RNA is copied using the virion-associated reverse transcriptase; ultimately dsDNA with long terminal repeats is made.

4. The DNA and integrase migrate to nucleus and the DNA is integrated into host DNA forming the **provirus**.

   The provirus remains in the host DNA.

   The rate of viral replication is regulated by the activity of the regulatory proteins (tat/rev, nef, etc).

   Tat upregulates transcription.

   Rev regulates transport of RNAs to cytoplasm.

   Co-infections (e.g., mycobacterial) stimulate the HIV-infected cells to produce more virus.

5. Transcription produces ss (+) RNAs, some spliced and some remain intact.
   - Spliced RNAs will be used as mRNA.
   - Whole RNA is used as genomic RNA.

6. Translation produces the proteins some of which are polyproteins that are cleaved by the HIV protease.

7. Assembly

8. Maturation/release of virus

HIV INFECTION

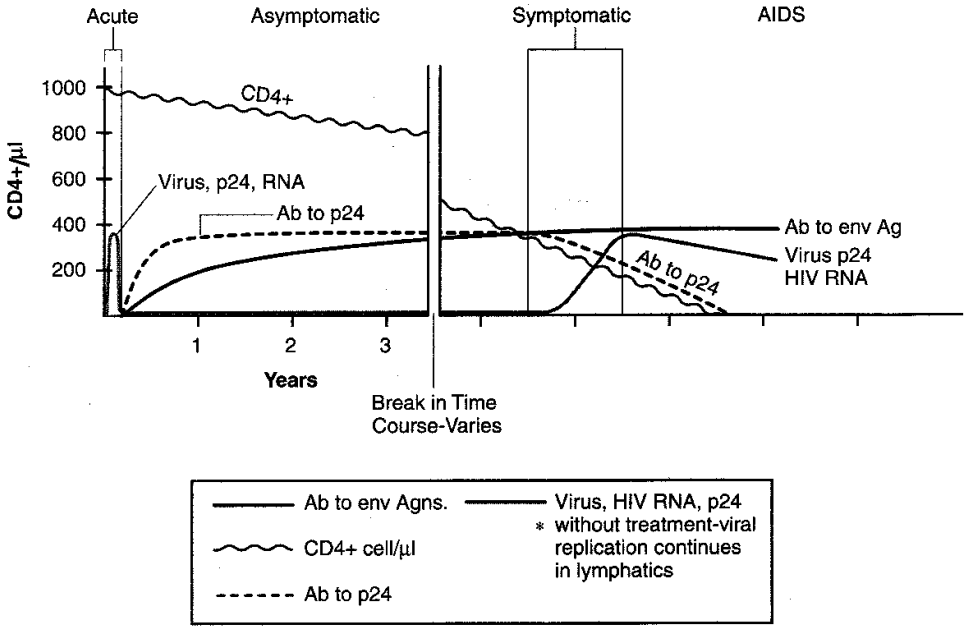


Figure I-5-26. Clinical Stages of HIV Infection

Table I-5-12. CDC Categories*

	Category A	Category B	Category C
CD4+ T cells/μL	(excludes conditions in B & C) **Acute** (primary) or **asymptomatic** HIV infection **Persistent generalized lymphadenopathy**	**Symptomatic** but not conditions in C Condition attributed to HIV infection (list below) or are indicative of a defect in cell-mediated immunity	**AIDS** defining conditions (See following list)
>500	A1	B1	C1
200–499	A2	B2	C2
<200	A3	B3	C3

AIDS = A3, B3, or C1–3

Acute phase has high level of viral production and mononucleosis-like symptoms: fever, sore throat, rash, malaise, lymphadenopathy, diarrhea, etc.

*1993 Revised Classification System for HIV Infection and Expanded Surveillance Case Definition for AIDS among Adolescents and Adults. MMWR December 18, 1992/41(RR-17)

CDC Category B (Symptomatic)

- Bacillary angiomatosis (disseminated Bartonellosis)
- Candidiasis (oral or persistent vulvovaginal)
- Cervical dysplasia or carcinoma *in situ*
- Constitutional sx (fever 38.5°C or diarrhea lasting >1 month)
- Hairy leukoplakia
- Idiopathic thrombocytopenic purpura
- Listeriosis
- Pelvic inflammatory disease (especially with abscess)
- Peripheral neuropathy

AIDS Defining Conditions (C)

- Encephalopathy, HIV-related
- Pneumonia, recurrent (leading cause of death)

Fungal Infections

- Candidiasis of esophagus, bronchi, trachea, or lungs
- Coccidioidomycosis, disseminated, or extrapulmonary
- Cryptococcosis, extrapulmonary
- Histoplasmosis, disseminated, or extrapulmonary
- *Pneumocystis carinii* pneumonia

Carcinomas

- Invasive cervical
- Kaposi's sarcoma; Burkitt's, immunoblastic, or primary CNS lymphoma

Viral Infections

- Cytomegalovirus retinitis (with loss of vision) or disease (other than liver, spleen, or nodes)
- Herpes simplex: chronic ulcer(s) (>1 month); or bronchitis, pneumonitis, or esophagitis
- Progressive multifocal leukoencephalopathy
- Wasting syndrome due to HIV

Parasitic Infections

- Cryptosporidiosis, chronic intestinal (>1 month)
- Isosporiasis, chronic intestinal (>1 month)
- Toxoplasmosis of brain

Bacterial Infections

- *Mycobacterium tuberculosis*, any site (pulmonary or extrapulmonary)
- *Mycobacterium avium* complex or *M. kansasii* or other species or unidentified species, disseminated or extrapulmonary
- *Salmonella* septicemia, recurrent

Diagnosis of HIV Infection

Screening

ELISA (most commonly done) to detect HIV antibodies in patient's serum. (Most tests include the antigens p24, p17, gp160, gp120, and gp41.)

- Anti-p24 is the first reliably detected antibody but declines as viral titers rise in late infection.
- Envelope antibodies rise more slowly but stay high at end. (Env antigens show major antigenic variation.)
- ELISA for p24 antigen useful early.

Confirmation (Using a Second Blood Sample)

- Western blot for antibodies specific for HIV (electrophoretically separated HIV antigens react with the patient's antibody; detection by enzyme-labeled anti-human IgG) or
- Immunofluorescence

HIV DNA PCR

- Qualitatitive to detect HIV infection in newborns of mothers are HIV+
- Quantitative HIV DNA PCR to determine viral load to assess treatment

Culture for HIV (with Antigen Detection in Culture)

- HIV infection in newborns whose mothers are HIV positive
- To assess drug resistance

Table I-5-13. HIV's Mechanisms of Immunologic Evasion

Characteristic	Function
Multiplication in lymphocytes and macrophages	Eliminates cell-mediated and antibody-mediated immunity
Nef and tat gene products down-regulate class I MHC expression.	Makes infected cells less susceptible to cytotoxic T-cell killing
Destruction of CD4 T cells	Elimination of immune enhancement response
Antigenic drift of the gp120 Heavy glycosylation of gp120	Evade antibody-mediated effector mechanisms

Table I-5-14. Negative Sense RNA Virus

Virus	RNA Structure	Virion-Associated Polymerase	Envelope	Shape	Multiplies in	Major Viruses
Paramyxovirus	ss(-) RNA Linear Non-segmented	Yes	Yes	Helical	Cytoplasm	Mumps Measles Respiratory syncytial Parainfluenza
Rhabdovirus	ss(-) RNA Linear Non-segmented	Yes	Yes	Bullet-shaped helical	Cytoplasm	Rabies Vesicular stomatitis
Filovirus	ss(-) RNA Linear Non-segmented	Yes	Yes	Helical	Cytoplasm	Marburg Ebola
Orthomyxovirus	ss(-) RNA Linear 8 segmented	Yes	Yes	Helical	Cytoplasm & nucleus	Influenza
Bunyavirus	ss(-) RNA Linear → Circular 3 segments Ambisense	Yes	Yes	Helical	Cytoplasm	California encephalitis La Crosse encephalitis Hantavirus
Arenavirus	ss(-) RNA Circular 2 segments 1 (-) sense 1 ambisense	Yes	Yes	Helical	Cytoplasm	Lymphocytic chorio-meningitis Lassa fever

Mnemonic for the ss(–) RNA viruses: Pairing Rats Fight Over Bunny's Area or Pain Results From Our Bunions Always.

You can remember these are the negative ones since fighting is a negative thing to do.

Note that all are enveloped, all have virion-associated polymerase, and all have helical nucleocapsids.

The oddballs are the last three:

The orthomyxoviruses are linear (ortho) but with eight (ortho/octo) segments, which is one of the reasons they can genetically "mix" it up. The orthomyxoviruses are also odd in that they replicate in both the nucleus and cytoplasm. The bunyaviruses are somewhat contortionists (circular): California playboy bunnies in a ménage à trois?

The arenaviruses have one negative sense and one ambisense strand of RNA.

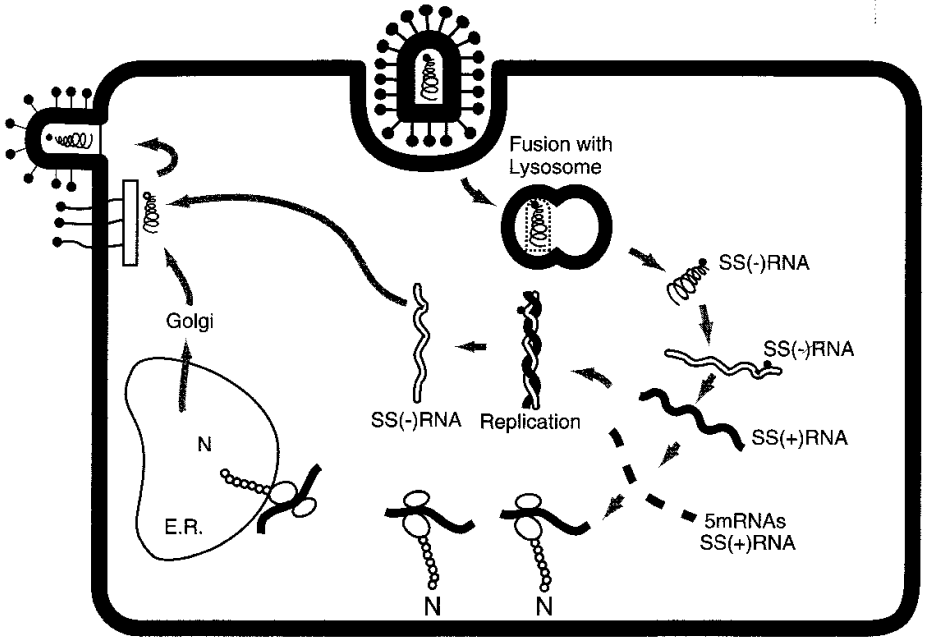


Figure I-5-27. Negative Sense RNA Virus Life Cycle

NEGATIVE SENSE RNA VIRUSES – DISEASES

Paramyxoviridae

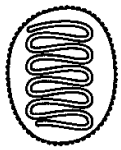


Figure I-5-28. Paramyxovirus

- ss(–) RNA strand
- Enveloped helical nucleocapsids

Parainfluenza virus

- Single HN glycoprotein, also fusion protein (F)
- Croup (laryngotracheobronchitis)
- Common cold, bronchitis

Mumps

- ss(–) RNA
- Single HN glycoprotein, also F protein
- Live vaccine
- Parotitis
- Pancreatitis
- Orchitis in adult males
- Meningoencephalitis

Measles (rubeola)

- ss(–) RNA
- H glycoprotein and fusion protein, no neuraminidase
- Measles:

  Presentation generally the three C's with photophobia:

  Cough, coryza, and conjunctivitis

  Koplik spots → maculopapular rash from the ears down → Giant cell

  Pneumonia (Warthin-Finkeldy cells)

  Rare complication: subacute sclerosing panencephalitis (chronic CNS degeneration)
- Live vaccine (single strain)

Respiratory syncytial virus

- ss(–) RNA
- No H nor N glycoproteins; only F protein
- Major cause of bronchiolitis and pneumonia in infants
- Common cold

Rhabdoviridae

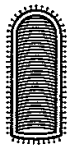


Figure I-5-29. Rhabdovirus

- ss(–) RNA
- Bullet shaped
- Enveloped

Rabies virus

- Negri bodies—intracytoplasmic inclusion bodies
- Inactivated vaccines; passive immunization
- Spread to humans by bites of rabid dogs; contact with bats
- Eastern U.S. reservoirs: foxes & raccoons; western U.S.: skunks

Vesicular stomatitis virus

Foot and mouth disease

Filoviridae

- ss(–) RNA
- Helical capsid with envelope
- Virion-associated RNA-dependent RNA polymerase

Marburg virus

Acute hemorrhagic fever, frequently fatal

Ebola virus

Acute hemorrhagic fever, frequently fatal

Orthomyxoviridae

Influenza

- ss(–) RNA
- Segmented (8)
- Enveloped nucleocapsids
- Separate H and N glycoproteins

Figure I-5-30. Orthomyxovirus

- Can lead to Guillain-Barré (A or B) or Reye's syndrome (primarily B)
- Inactivated vaccine, H1N1 and H3N2
- Influenza A & B (and many other viruses, most notably HIV) undergo **genetic drift** = slight changes in antigenicity due to mutations (in influenza responsible for epidemics)
- Influenza A has rare **genetic shift** (genetic reassortment) - major changes from new combinations of RNA segments or recombination between the segments in co-infections causing new pandemics

Bunyaviridae

- Segmented single (–) RNA
- Enveloped helical capsids (circularize) ambisense
- California group

California encephalitis and LaCrosse encephalitis viruses

- Mosquito-borne
- Young (<15 years) have more severe cases

Hanta virus (Sin Nombre)

- Hanta virus pulmonary syndrome (cough, myalgia, dyspnea, tachycardia, pulmonary edema and effusion, and hypotension [mortality 50%])
- High endemic area: Four Corners region (UT, AZ, NM, CO), but all of North America
- Associated with rodent feces

Arenaviridae

- Segmented RNA (2 pieces)
- ss(–) RNA, ss (ambisense) RNA
- Enveloped
- Helical

Lymphocytic choriomeningitis virus

- Influenza-like with meningeal signs
- Imported from South America

Lassa fever virus

- West Africa
- Hemorrhagic fever
- 50% fatal

DOUBLE-STRANDED RNA VIRUS–DISEASE

Table I-5-15. Double-Stranded RNA Viruses

	RNA Structure	Virion-Associated Polymerase	Envelope	Shape	Major Viruses
Reovirus	Linear, dsRNA 10–11 segments	Yes	Naked	Icosahedral double shelled	Reovirus Rotavirus

Reoviridae

Reoviruses

Upper respiratory tract infections

Figure I-5-31. Reovirus

Rotaviruses

- GI tract infection especially <2 years old, a prolonged diarrhea
- Major cause of infant mortality worldwide

ONCOGENIC VIRUSES

Definitions

Malignant Transformation of Cells

- Dedifferentiation
- Loss of growth control
- Immortalization
- Appearance of new surface antigens ("T" antigens)

Provirus

Viral DNA inserted into host DNA

Oncogenes

Genes with the potential to cause malignant transformation

Cellular Oncogenes (abbreviated c-onc)

These are normal cellular genes whose products control regulation of cell growth and division (e.g., kinases, growth factors and their receptors, G proteins and nuclear regulatory proteins).

Viral Oncogenes (abbreviated v-onc)

Genes carried by certain viruses causing cancer. Viral oncogenes are homologs of cellular oncogenes.

Tumor Suppressor Genes

These genes suppress, or constrain, cell growth and replication.

Major Concepts of Tumorigenesis

Mutation of a c-Oncogene or Tumor Suppressor Gene

- Mutation in one of these control genes may result in unregulated growth of cells.
- Example of mutated oncogene—ras
- Retinoblastoma (Rb) is an example of mutation in tumor suppressor gene.

Dosage Effects

- Oncogenes in amplified DNA—increased number of copies results in overexpression of gene.
- **Translocation**, which links an oncogene with a more active enhancer and/or promoter resulting in overexpression (Burkitt's lymphoma).
- Provirus **insertional mutagenesis**—for example, a retrovirus with its very active transcriptional promoter/enhancer region, the LTR (long terminal repeat) may integrate (insert) near a cellular oncogene. This is one of the mechanisms by which retroviruses that do not have v-onc cause carcinoma.

- Infection with a virus carrying a v-onc: e.g., infection with a retrovirus carrying viral oncogenes such as src. The gene was probably picked up by a provirus inserted near a cellular oncogene picking up copies of c-onc. Viral progeny then contain the new oncogene now called v-onc. When a new cell is infected with the recombinant virus, the oncogene is now under the transcriptional control of the viral enhancer/promoter.
- Interaction between the products of oncogenes and tumor suppressor genes. Proteins E6 and E7 of the human papilloma virus combine with and inactivate the p53 and p110 (Rb), respectively.

Specific Viruses Associated with Human Cancers

EBV

- Burkitt's lymphoma, nasopharyngeal, and thymic carcinoma
- BL occurs only in malarial regions; the plasmodia are thought to produce a slight immunosuppression.
- EBV stimulates B-cell replication and eventually, if a translocation of *c-myc* to the DNA region where genetic rearrangements involved in antibody synthesis occurs, BL develops.

Chronic HBV

Primary hepatocellular carcinoma

Chronic HCV

Primary hepatocellular carcinoma

HPV

- Cervical carcinoma
- Mechanism: inactivation of tumor suppressor gene

HTLV-1

- CD4+ T-cell leukemia/lymphomas
- Provirus insertion or capture

PRION DISEASES

Table I-5-16. Prion Diseases

Disease	Infectious agent	Host	Comments
Kuru	Prion	Human	Subacute Spongiform Encephalopathy (SSE); Fore Tribe - New Guinea; cannibalism
Creutzfeldt-Jakob Disease (and variant)	Prion	Human	SSE Genetic predisposition. Ingestion of infected cow brains
Gerstmann-Straussler	Prion	Human	SSE
Fatal Familial Insomnia	Prion	Human	SSE
Scrapie	Prion	Sheep	SSE—scraping their wool off on fences

Table I-5-17. Slow Conventional Viruses (Viruses)

Disease	Infectious agent	Host	Comments
Measles SSPE	Virus	Human having had measles	Subacute sclerosing panencephalitis
AIDS dementia	HIV	Human	Dementia
PML	JC Virus	Human	Progressive multifocal leukoencephalopathy

VIRAL GENETICS

Phenotypic Mixing

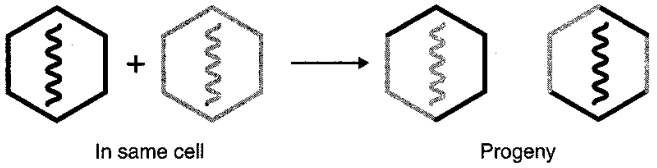


Figure I-5-32. Phenotypic Mixing

- Related viruses coinfect cell (virus A and virus B).
- Resulting proteins on the surface are a mixture capsid of AB around nucleic acid of either A or B.

Phenotypic Masking

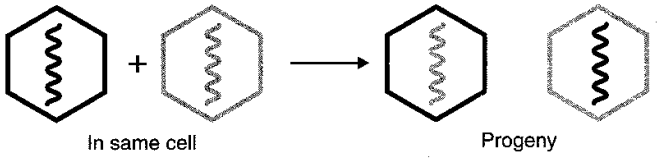


Figure I-5-33. Phenotypic Masking

- Related viruses coinfect cell (virus A and virus B).
- Capsid of proteins of virus A form around nucleic acid of B.

Complementation

- Two related defective viruses infect the same cell. If they are defective in different genes, viral progeny (still with mutated DNA) will be formed.

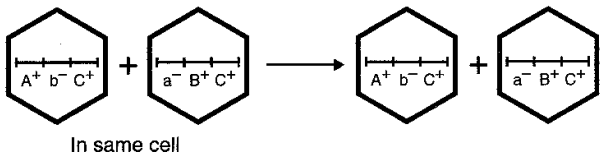


Figure I-5-34. Complementation: Mutations in Different Genes

If they are defective in the same gene, no progeny will be formed.

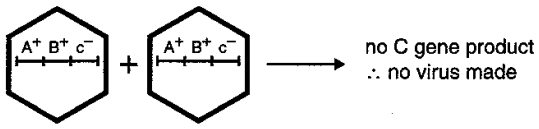


Figure I-5-35. Complementation: Mutations in Same Genes

- Coinfection of hepatitis B and D is a clinical example of complementation where HBV supplies the needed surface antigen for hepatitis D.

Genetic Reassortment = Genetic Shift

- Two different strains of a segmented RNA virus infect the same cell.
- Major new genetic combinations are produced through "shuffling," resulting in stable and dramatic changes.

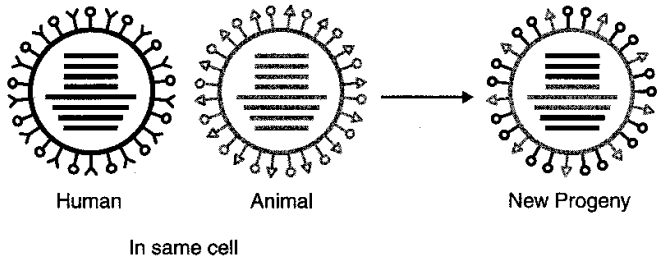


Figure I-5-36. Genetic Reassortment in Influenza A

Genetic Drift

- Minor antigenic changes from mutation
- Occurs in many viruses, particularly RNA ones
- Most noted in HIV and influenza

Viral Vectors

- Recombinant viruses are produced that have combinations of human replacement genes with the defective viral nucleic acid.

Chapter Summary

The virus structure consists of a core of RNA or DNA associated with structural proteins and perhaps key enzymes. This is called the nucleocapsid and constitutes a naked capsid virus. The nucleocapsid plus host membranes, including viral-specified glycoproteins, make up an enveloped virus.

The various viruses have characteristic sizes and shapes, as illustrated in Figure I-5-4.

The first step in viral replication is attachment. This is accomplished by the recognition of specific receptor sites on the host cell. This is followed by replication, release of the nucleic acid core (uncoating), and then macromolecular synthesis. Finally, new virus particles are assembled and released.

The eclipse period is the time it takes to replicate the first viral particles intracellularly. The latent period is the time between infection and the first release of virus from the infected cell.

Viruses are spread like any other pathogen. Arboviruses are those borne by arthropods, most commonly mosquitoes but also ticks, biting midges, and sandflies. Most arboviruses belong to the Togavirus, Flavivirus, or Bunyavirus families.

Virus attachment is mediated by an interaction between specific receptor sites on the host cell and either a specific viral surface glycoprotein on enveloped viruses or a specific surface protein on naked viruses.

Heat, detergents, or organic solvents inactivate enveloped (but not naked) viruses by destroying the lipid envelope.

The distribution of the required host receptors determines the species and tissue specificity for infection by a specific virus. Table I-5-1 summarizes the relationship among four important viruses, their targeted cells, and the cell receptors used for attachment by each of these viruses.

Once attached, the virus may invade the host cell by receptor-mediated endocytosis, by uptake via coated pits, or by the fusion of a viral envelope with the cell membrane.

The initial chore facing the invading virus is to synthesize protein that will replace normal host-cell function with those essential for viral survival and replication. To do so, the virus must use mRNA that will be recognized and used by the host cell's ribosomes.

Double-stranded DNA viruses directly transcribe mRNA using the negative strand as a template.

Retroviruses use their positively stranded RNA genome as templates to reverse transcribe double-stranded DNA using an RNA-dependent DNA polymerase (reverse transcriptase). The negative DNA strand is then used to transcribe mRNA.

Positively stranded RNA viruses can use their genome directly as mRNA.

Negatively stranded RNA viruses make the complimentary positive strand for use as mRNA.

Once reproduced, the naked viruses lyse the host cell. Enveloped viruses acquire host-cell membrane as an outer surface as they leave the cell.

The various patterns of viral infection are shown in Figure I-5-10 and Table I-5-5. They may be abortive, resulting in no infection; cytolytic, generally resulting in an acute infection; or persistent, resulting in a chronic infection, latent with possible later emergence of disease, or transforming, resulting in cancer.

(Continued)

Chapter Summary (*continued*)

Figure I-5-11 summarizes the ages at which various viral diseases may be expected to occur.

Immune defense against viruses utilizes the innate and adaptive pathways in a fashion similar to that used for protection from bacterial infection. However, a component of the innate sytem, interferon, is of unique importance as a protective mechanism against viral infection. Interferon is produced and released by virus-infected cells and is transmitted to noninfected cells where it inhibits nascent viral protein synthesis (Figure I-5-12).

Viral vaccines are developed using killed viruses, attenuated viruses (treated to lose virulence), and viral components. The properties and uses of each type of vaccine are summarized.

The responsible viral family, molecular features, transmission mode, disease characteristics, mortality rates, and diagnostic techniques associated with the hepatitis A, B, C, D, and E viruses are summarized in Table I-5-7. The characteristics of infection by the hepatitis B virus are discussed in greater detail on the following page and illustrated in Figure I-5-13.

The medically important DNA viruses include the Parvoviruses, Papovaviruses, Adenoviruses, Herpes viruses, Poxviruses, and the Hepadnaviruses. The properties and associated disease states of important members of each of these families are summarized in Tables I-5-8 and -9, Figures I-5-14, -15, -16, -17, and -18, and the accompanying text.

The medically important RNA viruses include the Calciviruses, Picornaviruses, Flaviviruses, Togaviruses, Coronaviruses, and Retroviruses. The properties and associated disease states of important members of each of these families are summarized in Tables I-5-10, -11, -12, -13, -14, and -15, Figures I-5-19, -20, -21, -22, -23, -24, -25, -26, -27, -28, -29, -30, and -31, and the accompanying text.

Table I-5-16 summarizes the characteristics of Kuru, Creutzfeldt-Jacob disease, Gerstmann-Straussler disease, fatal familial insomnia, and scrapie—all prion-associated diseases.

Table I-5-17 summarizes the slow effects of three conventional virus diseases (not prion). These are subacute sclerosing panencephalitis (SSPE) caused by latent measles virus, HIV-induced dementia, and progressive multifocal leukoencephalopathy (PML) provoked by the JC virus.

Natural genetic modification of viruses can occur by genetic mixing, phenotypic masking, complementation, genetic re-assortment (genetic shift), and genetic drift. In addition, the genetic characteristics of viruses can be altered by recombinant technology.

Review Questions

1. Three genetically distinct and fairly stable serotypes have made this virus's vaccines so successful that worldwide eradication of this virus is anticipated in the next 5 to 10 years.

   A. Adenovirus
   B. Herpes virus
   C. Influenza virus
   D. Measles
   E. Poliovirus

2. Roommates of a 19-year-old college student become alarmed when he does not get up to go to swim practice in the morning and they are unable to wake him for his 11 AM class (he had complained of a headache and not feeling well the night before). The rescue squad finds a febrile, comatose young man with a petechial rash. In the emergency room, Kernig and Brudzinski's signs are present. No papilledema is seen so a spinal tap is done. Protein is high, glucose low. CSF WBC count is 9,000 (mainly PMNs) with few RBCs. The characteristics of the most likely causative agent are

   A. An enveloped dsDNA virus
   B. A naked (+) ssRNA virus
   C. A Gram-negative bacillus with a polyribitol capsule
   D. A Gram-negative, oxidase-positive diplococcus
   E. A Gram-positive, lancet-shaped alpha-hemolytic diplococcus

3. Serologic test results from a hepatitis patient reveal: anti-HBc positive, HBsAg positive, and anti-HBs negative. The correct interpretation of the patient's status is

   A. No longer contagious
   B. Immune to hepatitis B virus
   C. Evidence of receiving hepatitis B vaccination
   D. Hepatitis B virus chronic carrier state
   E. Impossible to have both surface antigen and core antibody positive

4. Two individuals become infected with HIV at approximately the same time. JT progresses rapidly to full-blown AIDS and dies within 3 years. AY is still asymptomatic after 10 years. One possible explanation is that the virus in JT is probably expressing a high amount of activity of which regulatory gene product?

   A. *vif*
   B. *vpr*
   C. *tat*
   D. *env*
   E. *pol*

5. The best prospects for treatment and cure of microbial diseases are always those unique factors of a pathogen's life cycle that can be altered without affecting the survival of the host's own cells. In HIV, one such therapeutic target would be the products of the *pol* gene, which codes for the reverse transcriptase unique to the retroviral life cycle. If it were possible to ablate expression of the HIV *pol* gene, what other aspect of the virus's life cycle would be directly altered?

   A. Transcription of proviral DNA
   B. Production of viral mRNA
   C. Integration of proviral DNA
   D. Nucleocapsid
   E. Viral maturation

6. Interferons inhibit viral growth primarily by affecting

   A. Host cytokine production
   B. Host protein synthesis
   C. Viral protein synthesis
   D. Viral transcription process
   E. Viral assembly and release

7. Transfection with the naked nucleic acid of which virus would result in active viral replication?

   A. Bunyavirus
   B. Coxsackie
   C. Poxvirus
   D. Retrovirus
   E. Rhabdovirus

8. A chronic infection of hepatitis B is defined as having demonstrated the presence of

   A. HBsAg for more than 6 months
   B. HBsAg for more than 3 months
   C. Antibody against HBsAg for more than 6 months
   D. Antibody against HBcAg for less than 3 months
   E. HBcAg for 3 months

9. Live, attenuated vaccines exist for human diseases caused by

   A. Hepatitis A and B viruses
   B. Hepatitis A and polioviruses
   C. Rubella and rubeola viruses
   D. Influenza viruses
   E. Rabies virus and rotavirus

10. A boy with bilateral swelling of his salivary glands is found to have an elevated serum amylase. The most likely viral causative agent

    A. Has equal amounts of adenine and thymine in its genome
    B. May cause complications at the time of acute disease or during convalescence
    C. Has multiple serotypes
    D. Is characterized by being helical, circular, single-stranded RNA and naked

11. A 5-year-old female presents with a fever and a generalized macular rash that is most dense on the scalp and trunk of the body. Several waves of lesions appear, one after another, and evolve rapidly into vesicles and then pustules over several days. The most likely disease and causative agent is

    A. Exanthem subitum due to cytomegalovirus
    B. Chickenpox due to the varicella-zoster virus
    C. Whitlow's infection due to herpes simplex virus type 1
    D. Herpetic gingivostomatitis due to the varicella-zoster virus
    E. Infectious mononucleosis due to the Epstein-Barr virus

12. Infection of appropriate cells with a composite virus made up of Coxsackie virus capsid components and poliovirus RNA would yield progeny which would

    A. Have the host cell range of Coxsackie virus
    B. Also be composite viruses
    C. Show phenotypic mixing
    D. Have a recombinant genome consisting of both Coxsackie and poliovirus
    E. Cross-react with Sabin-vaccine–induced antibodies

13. Positive-stranded viral RNA

    A. Replicates most commonly through a double-stranded DNA intermediate
    B. Binds to ribosomes and can be translated
    C. Requires a special polymerase in the virion
    D. Is not infectious
    E. Replicates without an intermediate

14. To design a vaccine against HIV infection, a logical goal would be to alter some native molecule or product of the virion in order to make it highly immunogenic. If you wished to prevent the attachment of the virus to helper T lymphocytes, which molecule or family of molecules might best be targeted?

    A. gp41
    B. gp120
    C. nucleocapsid protein
    D. p17
    E. p24

15. How do the oncogenes of the human papilloma viruses act?

    A. They act as tumor suppressor genes
    B. They function identically to cellular oncogenes
    C. They kill the infected cell when upregulated
    D. We don't know how they act because they are present in every cell of every individual and yet only some become cancerous
    E. They code for early viral proteins, leading to malignant transformation

16. To which of the following viruses is hepatitis A most closely related genetically?

    A. Hepatitis B
    B. Poliovirus
    C. Measles
    D. Rubella virus
    E. Influenza

17. A naked protein similar in amino acid sequence to a normal human protein, but which has no nucleic acid associated with it, can cause

    A. AIDS dementia
    B. Mumps meningitis
    C. Progressive multifocal leukoencephalopathy
    D. Subacute sclerosing panencephalitis
    E. Subacute spongiform encephalopathy

18. A 37-year-old ambitious executive for a local Health Maintenance Organization comes to your office because he has developed multiple blister-like lesions on his penis over the last 1–2 days. They are somewhat painful, and he is worried that he has AIDS. He denies homosexuality and intravenous drug abuse and had an HIV test prior to his marriage 3 years ago. He reports several similar episodes several years ago when he worked as a photographer in Nepal. He was never told what they were, and they resolved over several days without any treatment. His physical examination is remarkable only for the presence of 6–8 vesicular lesions 3–4 mm in diameter on the glans of the penis. There is no crusting, drainage or bleeding. The lesions are moderately tender and there is mild inguinal adenopathy bilaterally. How does the causative agent produce its messenger RNA?

    A. By producing a positive sense intermediate
    B. By direct translation from the genome
    C. By transcription from proviral DNA
    D. By producing a negative sense intermediate
    E. By transcribing the genomic DNA
    F. By producing a double-stranded DNA intermediate
    G. The genomic RNA is used directly on the ribosomes

19. The Tzanck test, which aids in the diagnosis of herpes simplex infection, is a search for

    A. Antibodies to herpes simplex 1 or 2
    B. Intracytoplasmic inclusion bodies
    C. Virus shedding from pustular lesions
    D. Multinucleated giant cells
    E. Immunofluorescence of infected cells
    F. Detection of viral RNA
    G. Koilocytic cells

20. In the U.S., a baby has the greatest chance of acquiring which virus *in utero*?

    A. Cytomegalovirus
    B. Hepatitis B virus
    C. Herpes simplex virus
    D. Respiratory syncytial virus
    E. Rubella virus

21. Which of these viruses has RNA for both its genome and replicative intermediate?

    A. Cytomegalovirus
    B. Hepadnavirus
    C. Retroviruses
    D. Togaviruses
    E. Poxvirus

22. What is the most common lab testing method for diagnosing infectious mononucleosis?

    A. The Monospot test to detect EBV-specific antibody
    B. An assay for Epstein-Barr nuclear antigen
    C. The presence of atypical lymphocytes in the blood establishes the etiology
    D. A test for heterophile antibody, which cross-reacts with antigens found on a variety of animal red blood cells
    E. A simple procedure is done to isolate EBV from saliva, blood, or lymphoid tissue

23. What virus is noted for genetic reassortment, which leads to major pandemics about once every 10 to 11 years?

    A. Adenovirus
    B. Herpes virus
    C. Human immunodeficiency virus (HIV)
    D. Influenza virus
    E. Poliovirus

24. What virus is noted for such a high incidence of genetic drift that more than one antigenic variant can be isolated from most infected individuals who have high viral titers?

    A. Adenovirus
    B. Herpes virus
    C. Human immunodeficiency virus (HIV)
    D. Influenza virus
    E. Poliovirus

25. A 19-year-old male college student reports sore throat and extreme fatigue following even normal non-taxing tasks like getting dressed and going down to breakfast. He tells you that he has been sick for several weeks, that he has been feverish and that his girlfriend now appears to be getting the same thing. His tonsils are inflamed with a white exudate adhering; cervical lymphadenopathy is prominent, as is splenomegaly. The most likely causative agent is

    A. ss DNA, naked icosahedral virus
    B. ds DNA, naked icosahedral virus
    C. ds DNA, enveloped complex virus
    D. ds DNA, enveloped icosahedral virus
    E. ds RNA, naked segmented virus
    F. ss RNA, segmented enveloped and helical virus
    G. (–) ss RNA, bullet-shaped helical virus
    H. (–) ss RNA, naked helical virus
    I. (+) ss RNA, naked icosahedral virus
    J. (+) ss RNA, enveloped icosahedral virus
    K. (+) ss RNA, enveloped diploid virus

26. Cataracts and patent ductus arteriosus in a newborn suggest *in utero* infection with what viral family?

    A. Adenovirus
    B. Paramyxovirus
    C. Parvovirus
    D. Picornavirus
    E. Reovirus
    F. Togavirus

27. What is the most dominant method of spread for measles?

    A. Animal bite
    B. Fecal-oral
    C. Fomite spread
    D. Respiratory droplet spread
    E. Sexual contact
    F. Transfusion or intravenous drug abuse
    G. Tick bite

28. How are human papilloma virus type 4 warts spread?

    A. Animal bite
    B. Fecal-oral
    C. Fomite spread
    D. Respiratory droplet spread
    E. Sexual contact

29. A 15-year-old member of the high school swim team notices painless, umbilicated cutaneous lesions on the toes. Large eosinophilic cytoplasmic inclusions are present in the affected epithelia. What is the most likely causative agent?

    A. Adenovirus
    B. B-19 virus
    C. Cytomegalovirus
    D. Herpes simplex virus
    E. Human papilloma virus
    F. Molluscum contagiosum virus
    G. Varicella-Zoster virus

30. A bone marrow transplant recipient becomes febrile and hypoxic and chest films demonstrate diffuse interstitial pneumonia. What is the most likely causative agent?

    A. BK virus
    B. Cytomegalovirus
    C. Herpes simplex virus
    D. Molluscum contagiosum virus
    E. Paramyxovirus
    F. Varicella-Zoster virus

31. A 6-month-old infant presents with painless verrucous growths on the laryngeal folds. What is the most likely causative agent?

    A. B-19 virus
    B. Cytomegalovirus
    C. Herpes simplex virus
    D. Human papilloma virus
    E. Molluscum contagiosum virus

Answers

1. **Answer: E.** Poliovirus has three serotypes, which do not change significantly from year to year or within a person. The World Health Organization (WHO) hopes to have it eradicated within the next five years. (Note the clue of multiple vaccines.)

2. **Answer: D.** The most likely causative agent here is a bacterium. Viral meningitis is usually mild and would not fit the CSF values. Both the age of the patient and the petechial rash suggest it is most likely to be *Neisseria meningitidis*, which is a Gram-negative diplococcus that is oxidase-positive. The overproduction of outer-membrane fragments is what leads to the petechial rash, even prior to antibiotic treatment.

3. **Answer: D.** The positive test for hepatitis B surface antigen indicates that the patient still has the hepatitis B virus and hence is still contagious. The presence of hepatitis B surface antigen and the absence of the surface antibody (anti-HBs) indicate either an acute HBV infection (if patient has had the disease for only a short time) or a chronic carrier state (if the hepatitis has been going on for many months). Because acute HBV is not a choice, choice D then becomes the correct answer. Choice B would be a right answer if HBsAg is negative. Core antibodies would not be present if the person is only vaccinated. Also, HBs antigen should not be present in a detectable amount from vaccination.

4. **Answer: C.** Products of *tat* and *rev* genes increase viral maturation and act to decrease the latent period (J.T. probably had a high expression of those genes). The vif gene product affects viral infectivity, and vpr gene products increase the efficiency of budding. Another possible difference is that A.Y. may lack the invasion gene, which codes a cellular co-receptor for HIV. *Env* codes for envelope antigen and *pol* codes for reverse transcriptase.

5. **Answer: C.** The *pol* gene codes both for reverse transcriptase and the integrase, which allow the linear proviral DNA to be integrated, apparently at a random site, into a chromosome in the host cell. Of the distractors, both choices A and B are accomplished using the host cell's RNA polymerase. Choice D is a function of the *gag* gene, and choice E is controlled by *tat* and *rev* genes.

6. **Answer: C.** Interferons interfere with virus multiplication by blocking translation of viral protein (by inhibiting viral mRNA and hence inhibiting viral protein synthesis). They do not inhibit transcription, assembly, or release. They also do not inhibit host-protein synthesis.

7. **Answer: B.** Two of the viruses can be eliminated because they are negative RNA viruses and require polymerase accompanying the RNA to replicate. These two are the *Bunyavirus* and the rhabdoviruses. Since the retrovirus RNA requires the reverse transcriptase to replicate, it also cannot start an infection with just the RNA. Because of the locale of replication of the one DNA virus in the list (Poxvirus replicates in the cytoplasm), it must also bring in an enzyme to replicate: a transcribing enzyme.

8. **Answer: A.** Presence of HBsAg (surface antigen to hepatitis B virus) for 6 months (not 3 months) is considered chronic. Students need to know the abbreviations used for hepatitis serology.

9. **Answer: C.** Hepatitis A vaccine is a killed vaccine, and B is a component vaccine. The three important, live viral vaccines are MMR (mumps, measles, or rubeola, and rubella or German measles). The adenovirus vaccine (not in the question) is a live vaccine that is NOT attenuated but is enteric-coated and given orally to reduce respiratory infections in military personnel. Other live viral vaccines are attenuated and include Sabin's polio, varicella, and yellow fever.

10. **Answer: B.** Complications do occur. Choice A is wrong because the agent is the mumps virus, which is RNA (not DNA), and thereby should have uracil instead of thymine. Only one serotype exists, explaining why one gets mumps only once. Choice D is wrong because the genome of the mumps virus is linear and not circular.

11. **Answer: B.** The clinical presentation is consistent with chickenpox caused by VZV. Exanthem subitum is caused by human herpes virus 6, not by CMV. Herpetic gingivostomatitis refers to herpes simplex type 1, not VZV. Students need to know the typical clinical presentations of Whitlow's and infectious mononucleosis.

12. **Answer: E.** This question requires you to understand viral replication and viral genetics quite well. The only nucleic acid in the composite parental virus is the RNA belonging to poliovirus. Thus, only poliovirus is made. The only role the Coxsackie virus would play in the infection is to bind to the host cell and stimulate the uptake of the composite virus. Once uncoating takes place, the Coxsackie components play no further role. A perfect poliovirus will have been made.

    So **choice A** is incorrect—the progeny will have the host-cell range of polio because that is what they'll be.

    Choice B is incorrect because there is no genetic material coding for the Coxsackie components, so you cannot get a composite.

    Choice C is incorrect because no capsid components of Coxsackie will be made; there can be no mixing.

    Choice D is incorrect because there was only one type of RNA; there can never be recombination.

    Choice E is correct because Sabin is a polio-specific vaccine, and poliovirus will be produced.

13. **Answer: B.** Positive-stranded viral RNA is infectious because it can bind to ribosomes and be translated and therefore can make its polymerase (a RNA-dependent RNA polymerase). It does not need to carry it in its virion. It replicates by making a negative template of RNA, and only HIV replicates through a DNA intermediate. All single-stranded RNA viruses require a replicative intermediate, making choice E false.

14. **Answer: B.** Gp120 is the surface antigen of HIV that mediates its attachment to CD4 lymphocytes. Gp41 is a transmembrane glycoprotein, and p24, p17, and nucleocapsid protein are all internal molecules, which would rarely be accessible to the immune response.

15. **Answer: E.** Our normal tumor-suppressor genes are "anti-oncogenes," which negatively regulate cell growth. The oncogenes of human papilloma viruses code for two early proteins: E6 and E7, which inactivate the tumor-suppressor genes of the infected cells, resulting in malignant transformation. Viral oncogenes are similar but *not identical* to cellular oncogenes. Expression of viral oncogenes may lead to malignant transformation but will not kill the infected cells. HPV oncogenes are not present in every cell and certainly not in every individual.

16. **Answer: B.** Hepatitis A is a picornavirus so that is the most likely choice. If you do not know the family but know that it is naked and positive RNA, you can eliminate hepatitis B because it is DNA. Measles, rubella, and influenza are all enveloped.

17. **Answer: E.** The definition fits a prion. Prions cause subacute spongiform encephalitis (e.g., Kuru, Creutzfeldt-Jakob disease, Gerstmann-Straussler disease, etc.). PML is cause by the JC virus. AIDS dementia is HIV. SSPE follows measles and perhaps is caused by hypermutated, defective forms of the virus.

18. **Answer: E.** The virus is HSV II, a herpesvirus, which are dsDNA viruses that use the mechanisms of our own cells to transcribe an RNA strand from their genomic DNA and use the transcribed RNA as a messenger RNA. Of the distractors: choice A is the technique used by the negative sense RNA viruses; choices B and G are used by the positive sense RNA viruses; choice C is used by the retroviruses; choice D is used during the genomic duplication of negative sense RNA viruses; and choice E is used in genomic replication by the retroviruses. Choice F would not produce RNA.

19. **Answer: D.** In the Tzanck test, a swab is taken from the exposed base of a lesion and observed microscopically by Giemsa staining for the presence of characteristic multinucleated giant cells. The Tzanck test is not an immunofluorescence test (**choice E**) or a test for patient antibodies (**choice A**) or a viral culture. (Both direct immunofluorescent staining of cells from lesions and viral culture with DFA confirmation are commonly used for diagnosis in large hospitals because the Tzanck test is not specific.) Herpes simplex virus produces intranuclear infection (thus **choice B** is not true), and the lesions are generally not pustular (**choice C**). **Choice F** is not true because herpesviruses are DNA viruses, and koilocytic cells (**choice G**) are hallmarks of human papilloma virus infection in PAP smears.

20. **Answer: A.** CMV is an extremely common virus and crosses the placenta oftentimes without causing obvious symptomology. Fortunately, Rubella, which is highly teratogenic particularly in early pregnancy, is generally prevented by routine vaccination in childhood or at least 16 weeks prior to pregnancy. Less than 5% of hepatitis B could possibly be *in utero.* HSV 2 will only cross the placenta if mom acquires Herpes for the first time during her pregnancy. RSV and other respiratory viruses will not. Other viruses that can cross the placenta include Coxsackie B and HIV.

21. **Answer: D.** Cytomegalovirus, Hepadna, and Poxviruses are all dsDNA viruses and not RNA. Retrovirus is an RNA virus but replicates through a dsDNA, so it also is not the correct answer. Toga is a positive RNA virus, which replicates through a negative RNA intermediate and has no DNA; therefore, it's the correct answer.

22. **Answer: D.** Monospot is the most commonly used test for the diagnosis of infectious mononucleosis caused by EBV. However, it does not detect EBV-specific antibody. It instead detects heterophile antibody, which is nonspecific in that it may be present in different organisms and individuals and it cross-reacts with many animal RBCs. Epstein-Barr nuclear antigen test is routinely run in the diagnosis of mononucleosis. Atypical lymphocytes are found in mononucleosis caused by EBV and CMV, but CMV is heterophile antibody–negative. Isolation of EBV, of course, can establish the diagnosis. However, the procedure is cumbersome and laborious, and would identify asymptomatic infected cases as well.

23. **Answer: D.** The segmented influenza viruses may undergo recombination with a similar animal virus. This leads to genetic change that negates everyone's prior immunity, leading to severe pandemics.

24. **Answer: C.** HIV. It is this genetic drift that makes it difficult for the body to fight off HIV and has complicated the development of an effective vaccine.

25. **Answer: D.** Both the symptomology, length of infection, and the epidemiological clues (college student, age 19, has given it to his girlfriend) strongly suggest that this is EBV, which is a herpesvirus.

    Choice A = parvo; **choice B** = adeno; **choice C** = pox; **choice D** = papova/hepadna because there's no distinction as to circular or partial ds DNA; **choice E** = reovirus; **choice F** = arena virus, bunya, and orthomyxo; **choice G** = rabies; **choice H** = none; **choice I** = calici or picorna; **choice J** = flavi and toga; **choice K** = retro.

26. **Answer: F.** The description fits congenital rubella, a togavirus, which is an enveloped positive RNA virus that is not segmented.

27. **Answer: D.** If you have any trouble, think about which of these viruses has respiratory symptoms (in this case, pneumonia).

28. **Answer: C.** Remember that type 4 strains cause plantar warts, and these are largely transmitted by shower room floors, towels, etc.

29. **Answer: F.** This describes the typical presentation of molluscum contagiosum, which is commonly acquired through small breaks in the skin in environments where moisture keeps the virus viable (swimming pools, showers).

30. **Answer: B.** CMV is the most common viral cause of death in bone-marrow transplant patients, causing an interstitial pneumonia.

31. **Answer: D.** Perinatal infection with human papilloma virus can cause infantile laryngeal warts.

Matching

A. Match disease with causative agent. Remember that choices may be used once, more than once, or not at all.

A. Adenovirus
B. Coronavirus
C. Coxsackie virus
D. Echovirus
E. Gerstmann-Straussler
F. Herpes 1
G. Marburg agent
H. Measles
I. Norwalk agent
J. Parvovirus
K. Rabies
L. Rotavirus
M. Rubella
N. Vaccinia
O. Varicella-Zoster

___1. A common cause of acute coryza, a usually afebrile upper respiratory infection causing inflammation of the nose, paranasal sinuses, throat, larynx, and trachea.

___2. The patient has experienced a low-grade fever and stiff neck for the past day. This morning he awakened with difficulty and his wife noted distinct personality changes. He experienced several seizures and was brought into the emergency department. A non-traumatic spinal tap yielded CSF fluid with lymphocytes and RBCs. A diagnosis of encephalitis was made.

___3. An epidemic of diarrheal disease has occurred at Lincoln Grade School. Twenty percent of the children have been ill enough to stay at home and three teachers and a custodian have also called in sick.

___4. Several children in a daycare center have developed papulovesicular erythematous lesions over the buccal mucosa and palate. The children had experienced fever, headache, and sore throat a few days preceding the appearance of the vesicular lesions. Similar lesions are noted on the hands and feet and in the diaper area in the very young.

___5. Over fifty percent of the boys in Scout Troop #27 have developed a febrile disease during the first week after their camp out on the lake. They all complain of sore throat and itchy eyes; their conjunctiva are inflamed. A few other members of the troop have conjunctivitis but no signs of pharyngitis.

___6. This 17-year-old child has been bothered with insomnia for the past several weeks. Her schoolwork has suffered and she has been having hallucinations. The onset of seizures has prompted a neurologic examination. The presence of elevated levels of gamma globulin in the spinal fluid prompts the neurologist to order an EEG. A diagnosis of subacute sclerosing panencephalitis is made. What earlier viral infection has she had?

___7. The patient complains of easy fatigability and insomnia. He is somewhat apathetic and disoriented. Aphasia and other signs of abnormal higher cortical functions are noted. A neurological consult examines the patient and suggests that he might have a subacute spongiform encephalopathy.

___8. The patient is a well-developed young man of 16 years of age who has been working on a combine crew this summer. He has a sudden onset of malaise and fever and complains of a global headache and a stiff neck and back. CSF exam reveals an elevated opening pressure, normal glucose, but elevated proteins. The predominating cell in the spinal fluid is a lymphocyte.

Answers to A

1. **B** Because the rhinoviruses are not here, you have to choose the coronaviruses.

2. **F** Herpes 1 is a major causative agent of encephalitis.

3. **I** Communal eating may lead to food contaminated by a food preparer with Norwalk virus. Characteristically, infants are less likely to be involved not having eaten the food, even though they may have attended the event.

4. **C** This is hand, foot, and mouth disease caused by Coxsackie A virus.

5. **A** Adenovirus

6. **H** Late sequela to measles (rubeola)

7. **E** This is prion disease. The only prion listed is Gerstmann-Straussler.

8. **D** The most common causative agent of aseptic meningitis is echovirus.

B. Match viral feature with agent.

A. Adenovirus
B. Rubella
C. Influenza A
D. Herpes 1
E. Human immunodeficiency virus
F. Human papilloma virus
G. Hepatitis B
H. Variola

___1. Genetic shift important to epidemiology

___2. Highest transmissibility rate by needle stick

___3. Oncogenic virus

___4. Self-coded envelope

___5. Teratogenic

Answers to B

1. **C** Shift is in influenza A, while drift also is notable in HIV.

2. **G** Hepatitis B is acknowledged to be more infectious than HIV.

3. **F** HPV has the strongest evidence for being carcinogenic. HIV is not considered directly oncogenic.

4. **H** Smallpox (variola) is the only one that makes its own envelope.

5. **B** Rubella is most noted for teratogenicity.

C. These are killers. If you can do these, you are in excellent shape! Write what you know about the virus, and then look on the list, rather than trying to do it in whatever order they have given you the characteristics.

Match features with viral types.

A. Icosahedral, enveloped, double-stranded DNA
B. Icosahedral, naked, double-stranded DNA
C. Icosahedral, naked, single-stranded DNA
D. Icosahedral, naked, positive single-stranded RNA
E. Icosahedral, enveloped, positive single-stranded RNA
F. Icosahedral, double-stranded, segmented DNA
G. Helical, naked, negative single-stranded RNA
H. Helical, enveloped, negative single-stranded RNA
I. Helical, enveloped, negative, single-stranded, segmented RNA

___1. The baby was born at the 31st week of gestation. It was small for gestational age and showed jaundice. Petechiae were noted and the spleen and liver were enlarged. The pathology department reported that placental examination revealed chorioamnionitis, villitis, and owl's eye intranuclear inclusion bodies.

___2. The Peace Corps worker was bitten by a camp dog while helping in the construction of a dam near the village of Urundan in Central Africa. The animal was caged and 4 days after the attack, died. The head was sent to a reference laboratory and Negri bodies were found in Ammon's horn.

___3. A 4-year-old child of Mexican immigrant parents was brought to the hospital with a temperature of 101°F, a cough coryza, conjunctivitis, and a characteristic exanthem on the buccal mucosa opposite the first and second upper molars. The spots resemble tiny grains of white sand surrounded by inflammatory areolae.

___4. A 16-year-old high school dropout develops urticaria and arthralgia. He reports that his urine is dark in color and his sclera are yellow. He has not felt well for the past week or so and has quit smoking because the cigarettes started to taste funny.

Answers to C

1. **A** Owls eye's inclusion bodies are found in cytomegalovirus, which is a Herpes virus. The viruses are DNA (double-stranded), enveloped, and icosahedral.

2. **H** Negri bodies are found in rabies, which is a negative single-stranded RNA virus, both helical and enveloped.

3. **H** Koplik spots are associated with measles, which is an enveloped, helical, negative, single-stranded RNA virus.

4. **A** Dane particles are the infectious particle of hepatitis B that are icosahedral, enveloped, double-stranded DNA viruses.

Microbial Genetics/ Drug Resistance

6

TERMINOLOGY

Polymerases

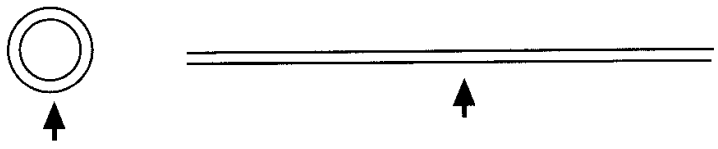
(major nucleic acid synthesizing polymerases)

_____-dependent _____ polymerase
(template) **(product)**

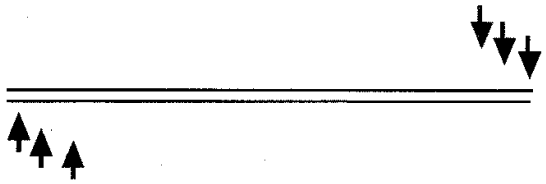
_____-dependent _____ polymerase
(template) (product)

Nucleases

Endonucleases—cleave the nucleic acid backbone in the middle of the nucleic acid strand. Restriction endonucleases (a.k.a., site-specific endonucleases) recognize specific base sequences and make specific breaks. Bacterial cells methylate their own restriction nuclease sites so that their enzymes do not break down their own DNA. Endonucleases are not present in high concentration in the cells.



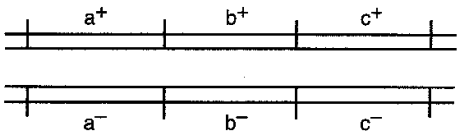
Exonucleases—remove end nucleotides sequentially from linear pieces of nucleic acid, ultimately totally breaking the piece of DNA down. Numerous exonucleases per cell.



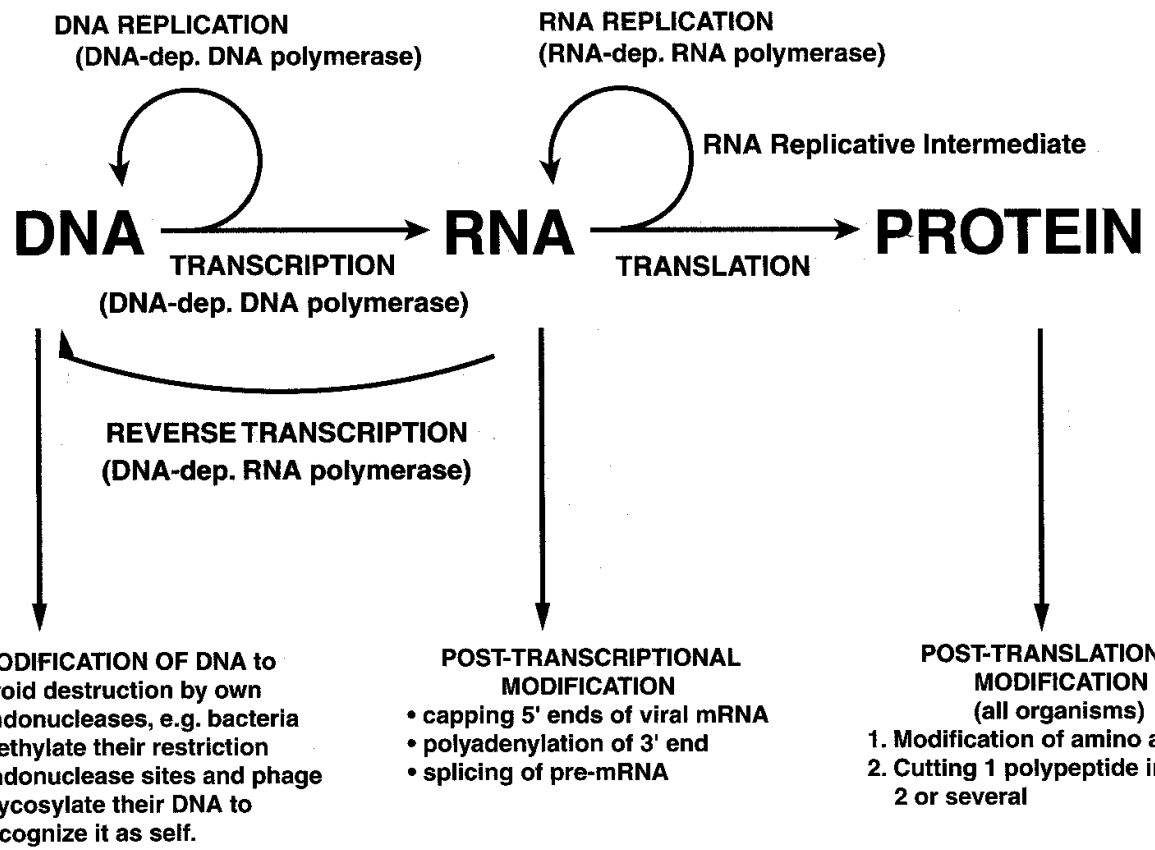
Alleles

Alternative forms of the same gene.

In the following two strands of DNA, *a*, *b*, and *c* are different genes but a^+ and a^- are different alleles.



THE FLOW OF GENETIC INFORMATION

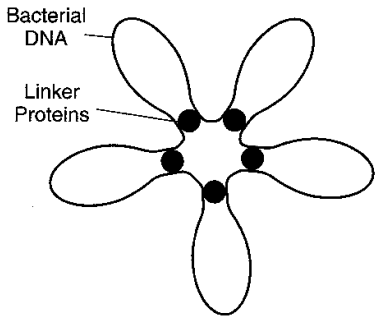

DNA REPLICATION
(DNA-dep. DNA polymerase)
RNA REPLICATION
(RNA-dep. RNA polymerase)
RNA Replicative Intermediate
DNA
TRANSCRIPTION
(DNA-dep. DNA polymerase)
RNA
TRANSLATION
PROTEIN
REVERSE TRANSCRIPTION
(DNA-dep. RNA polymerase)
MODIFICATION OF DNA to avoid destruction by own endonucleases, e.g. bacteria methylate their restriction endonuclease sites and phage glycosylate their DNA to recognize it as self.
POST-TRANSCRIPTIONAL MODIFICATION
• capping 5' ends of viral mRNA
• polyadenylation of 3' end
• splicing of pre-mRNA
POST-TRANSLATIONAL MODIFICATION
(all organisms)
1. Modification of amino acids
2. Cutting 1 polypeptide into 2 or several

THE BACTERIAL GENETIC MATERIAL

Three different types of DNA may be found in a bacterial cell: bacterial DNA, plasmid DNA, or bacteriophage DNA.

Bacterial Chromosome (Genome)

- Most bacteria have only **one chromosome but often multiple copies** of it in the cell.
- Most bacterial chromosomes are a **large covalently closed, circular DNA molecule** (about 1,000 times the diameter of the cell).
- The chromosome is **organized into loops** around a proteinaceous center. A single-stranded topoisomerase (1 nick) will relax only the nicked loop, allowing DNA synthesis or transcription.
- Most have **around 2,000 genes.** (*E. coli* has about 4,500 kbases.)
- All **essential genes** are on the bacterial chromosome.



Plasmids

- Are **extrachromosomal genetic elements** found in bacteria (and eukaryotes)
- Are generally covalently closed, **circular DNA**
- Are **small** (1.5–400 kB)
- Can replicate autonomously in bacterial cells
- One subclass of plasmids, called **episomes**, may be integrated into the bacterial DNA. Episomes have insertion sequences matching those on the bacterial chromosome.
- Plasmids carry the genetic material for a variety of genes, e.g., the fertility genes directing conjugation (*tra* operon), many of the genes for antibiotic resistance, and most bacterial exotoxins.

Bacteriophage (= phage = bacterial virus) Genome

- **Stable pieces of bacteriophage DNA** may be present in the bacterial cell.
- These are **generally repressed temperate phage DNA inserted into the bacterial chromosome (called a prophage)**.
- **Besides the repressor protein**, this prophage DNA may also direct synthesis of another protein. Most notable are gene products that make bacteria more pathogenic. This enhanced virulence is called **lysogenic conversion.**

INSERTION SEQUENCES/TRANSPOSONS

Transposons (Tn) and Insertion Sequences (IS)

- Are mobile genetic elements (DNA) that can move themselves or a copy from one molecule of DNA to another ("jumping genes")
- Are found in eukaryotic and bacterial cells and viruses
- Have sequences of indirect repeats of bases on each end
- Have at least one gene for a transposase (enzyme[s] involved in the "movement")
- Create additional mutations with their insertion in another totally unrelated gene
- May insert anywhere the transposase recognizes the specific sequence of nucleotides.
- Insertion creates direct repeats on each side of the transposon/IS.

Phage-Coded Pathogenic Factors or Lysogenic Conversion

O = O antigen of *Salmonella*

B = Botulinum toxin

E = Erythrogenic exotoxins of *S. pyogenes* (SPE-A, -B, and -C)

D = Diphtheria toxin

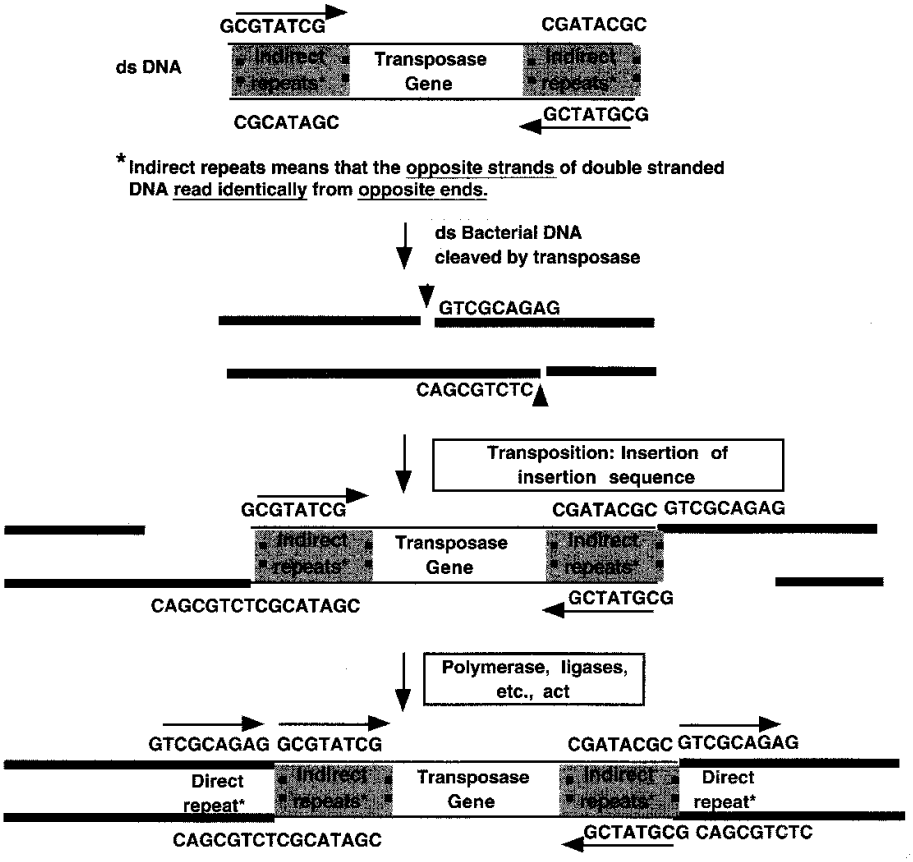
(OBED: a little bit pregnant with phage!)

Insertion Sequences (IS)

Minimal transposable elements (**A, below**)

- Have just the one gene for transposase.
- Have the terminal indirect repeats.
- Have promoters and transcription and translation-termination signals.

Insertion Sequence Showing Terminal Indirect Repeats* + Genes for Transposition



*Indirect repeats means that the opposite strands of double stranded DNA read identically from opposite ends.

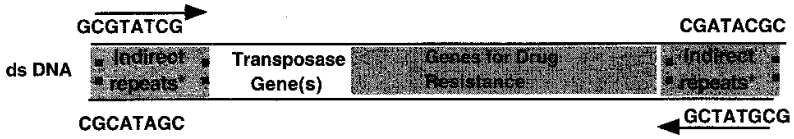
*Direct repeat means that the same strand read from the same direction is identical. It is created because a homologous strand was separated over a short space and then each strand used as a template.

Transposons (Tn)

Insertion sequences plus at least one other gene

- Include some genes for drug resistance
- Transposons play an important role in building multiple drug resistance plasmids. They also (but not as frequently) may move to the chromosome.

Transposons have additional genes, e.g., drug resistance genes



REARRANGEMENT OF DNA WITHIN A BACTERIUM

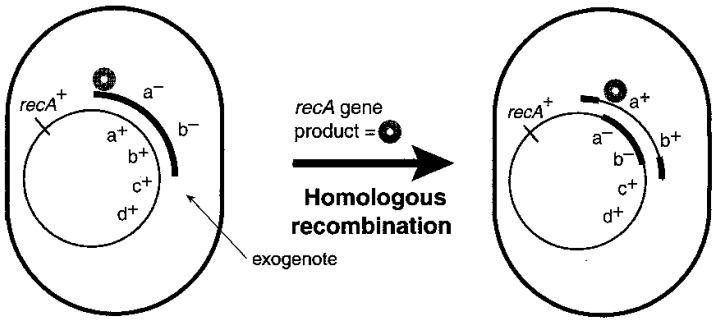
Homologous Recombination

Homologous recombination is a gene exchange process that may stabilize some genes introduced by transformation, conjugation, or transduction.

Imported bacterial DNA (transferred into a cell by transformation, conjugation or transduction) **is on short linear pieces of DNA called exogenotes.** Most **linear DNA is not stable in cells because it is broken down by exonucleases.**

Homologous recombination produces an "exchange" of pieces of DNA between the linear piece of DNA and it is near a homologous region on the stable (circular) bacterial chromosome. **Homologous recombination requires:**

- **Several genes worth of homology or near homology** between the DNA strands.
- **A series of recombination enzymes/factors** coded for by the recombination genes ***recA***, *recB*, *recC*, and *recD* (with *recA* generally an absolute requirement).

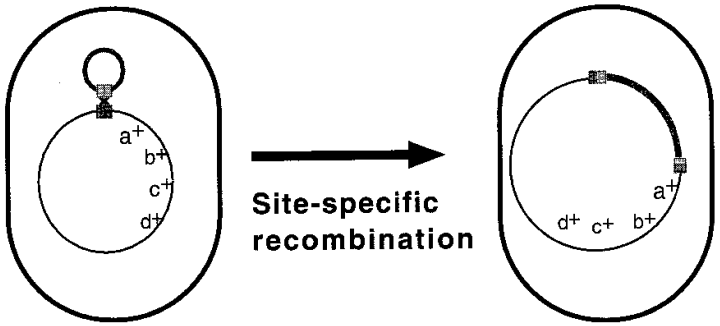


Genes ending up on the linear piece of DNA are lost.
Those on the circular molecule become part of the cell's **permanent** genetic make up.

Site-Specific Recombination

Site-specific recombination is the integration of a DNA molecule into a DNA with which it has no homology except for a small site on each DNA (called an attachment, integration or insertion site).

- **Requires restriction endonucleases** and **restriction endonuclease sites** on each DNA (often named an integration site or attachment site but basically insertion sequences) but DNA synthesis is not required.
- Since this process integrates rather than exchanging pieces of DNA, **the end result is a molecule the sum of the two original molecules.**



▩ = Integration (a.k.a. insertion) sites

Three major roles of site-specific integration

- **Integration of a fertility factor** to make an Hfr cell
- **Integration of temperate phage DNA** into a bacterial chromosome to create a prophage
- **Movement and insertion of transposons (transposition is the name of site-specific integration of transposons)**

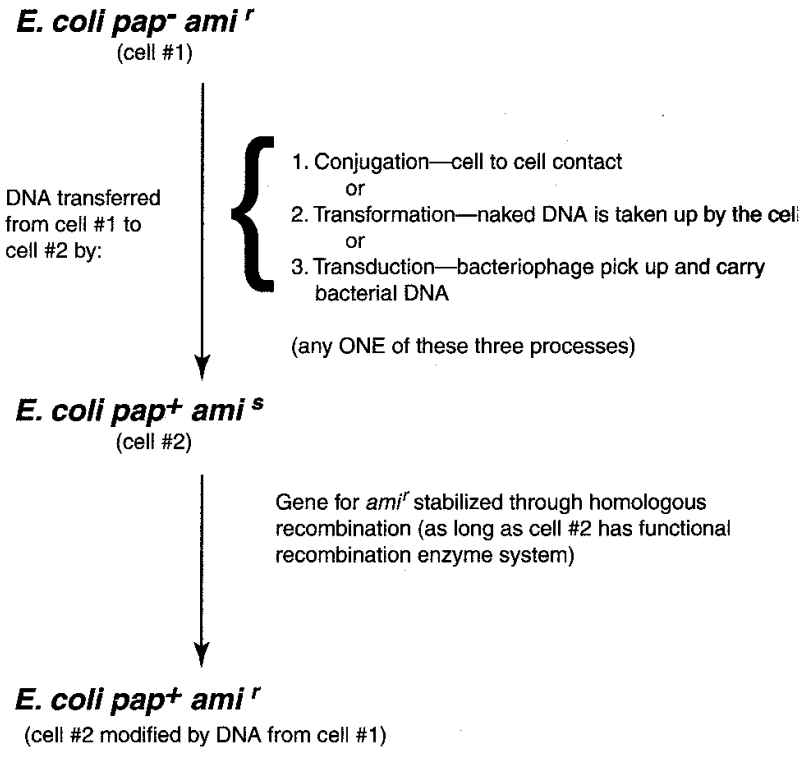
GENE TRANSFER

Overview

Bacterial reproduction is asexual so progeny are identical to parent cell with only rare mutations.

How do you get new genetic combinations in bacteria?

Answer: Gene transfer followed by stabilization of genes (recombination).

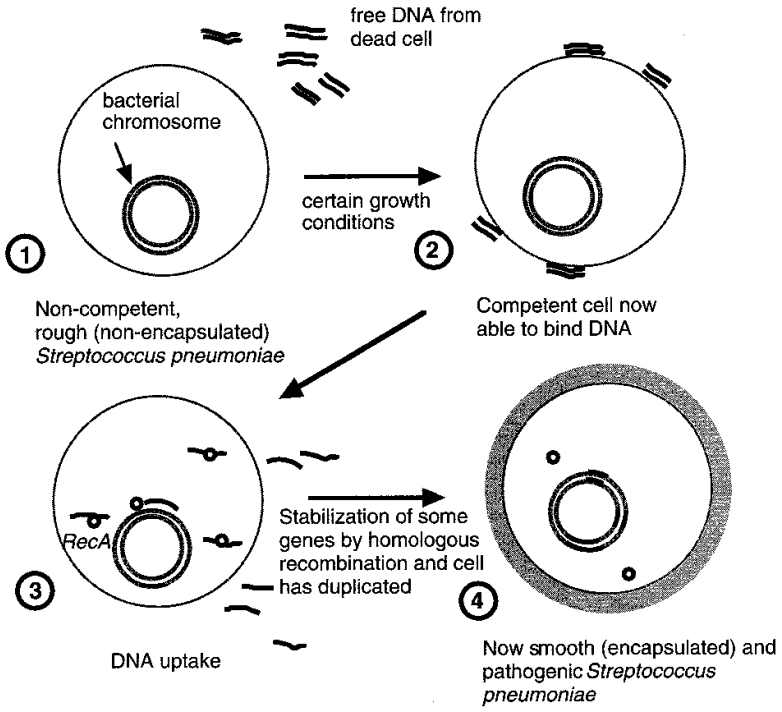

E. coli pap⁻ ami r
(cell #1)
DNA transferred from cell #1 to cell #2 by:
1. Conjugation—cell to cell contact
or
2. Transformation—naked DNA is taken up by the cell
or
3. Transduction—bacteriophage pick up and carry bacterial DNA
(any ONE of these three processes)
E. coli pap+ ami s
(cell #2)
Gene for ami r stabilized through homologous recombination (as long as cell #2 has functional recombination enzyme system)
E. coli pap+ ami r
(cell #2 modified by DNA from cell #1)

- Now pap^+ (initially linked to ami^S) is linked to ami^r instead, producing a new combination of genes and more significantly, a cell that can cause pyelonephritis and is amikacin resistant.
- (Could also have yielded *E. coli* that was pap^- ami^S or pap^- ami^r or the cell could have stayed pap^+ ami^S.)

Transformation

Transformation is the uptake of naked DNA from the environment by competent cells.

- Cells become competent (able to bind short pieces of DNA to the envelope and import them into the cell) under certain environmental conditions (which you do not need to know).
- DNA (released from dead cells) is taken up.
- Newly introduced DNA is generally linear, homologous DNA from same type of cell but perhaps one that is genetically diverse.
- The steps of transformation of a non-encapsulated *Streptococcus pneumoniae* are shown below.



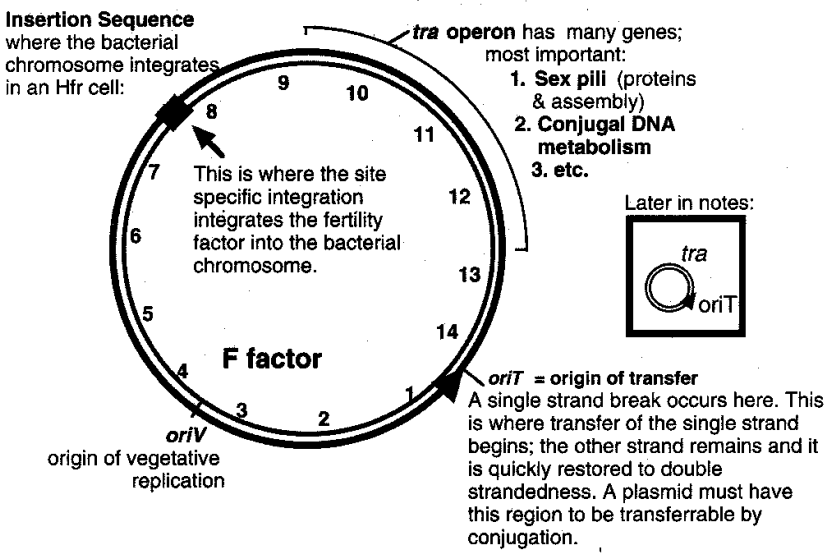
Conjugation

Conjugation is gene transfer from one bacterial cell to another involving direct cell-to-cell contact.

- **Fertility factors control** conjugation
- Sex pili (genes on F factor) play a role in establishing cell-to-cell contact.
- A single strand (or a portion thereof) of the double helix of DNA is transferred from the donor (or male) cell to the recipient or female cell.
- Bacterial genes transferred in by conjugation have to be stabilized by homologous recombination (i.e., in an Hfr × F^- cross). Plasmid genes transferred by conjugation circularize and are stable without recombination.
- Conjugation with recombination may produce new genetic combinations.

Donor (Male) Cells

- ALL have fertility plasmids known as F factors. F factors have a series of important plasmid "fertility" genes called the transfer or *tra* region which code for:
  - sex pili
  - genes whose products stabilize mating pairs
  - genes which direct conjugal DNA transfer, and other genes.
- Have a region called *oriT* (origin of transfer) where a single strand break in the DNA will be made and then *oriT* begins the transfer of one strand of the double helix.
- Many have insertion sequences where the plasmid can be inserted into the bacterial chromosome combining to make one larger molecule of DNA.
- A genetic map of an F factor is shown below.

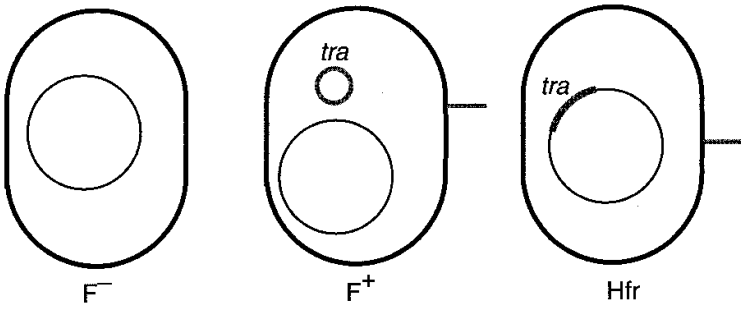

Insertion Sequence where the bacterial chromosome integrates in an Hfr cell:
***tra* operon** has many genes; most important:
1. **Sex pili** (proteins & assembly)
2. **Conjugal DNA metabolism**
3. **etc.**
This is where the site specific integration integrates the fertility factor into the bacterial chromosome.
Later in notes:
tra
oriT
F factor
1
2
3
4
5
6
7
8
9
10
11
12
13
14
***oriT* = origin of transfer**
A single strand break occurs here. This is where transfer of the single strand begins; the other strand remains and it is quickly restored to double strandedness. A plasmid must have this region to be transferrable by conjugation.
oriV
origin of vegetative replication

- Donor cells in which the **fertility plasmid** is in its **free state** are called **F^+ cells.**
- Donor cells in which the **fertility factor has inserted** itself into the bacterial chromosome are called **Hfr cells.**

Recipient (Female) Cells: F^- Cells

- Recipient cells lack fertility factors and genes.
- In every cross, one cell must be an F^- cell.

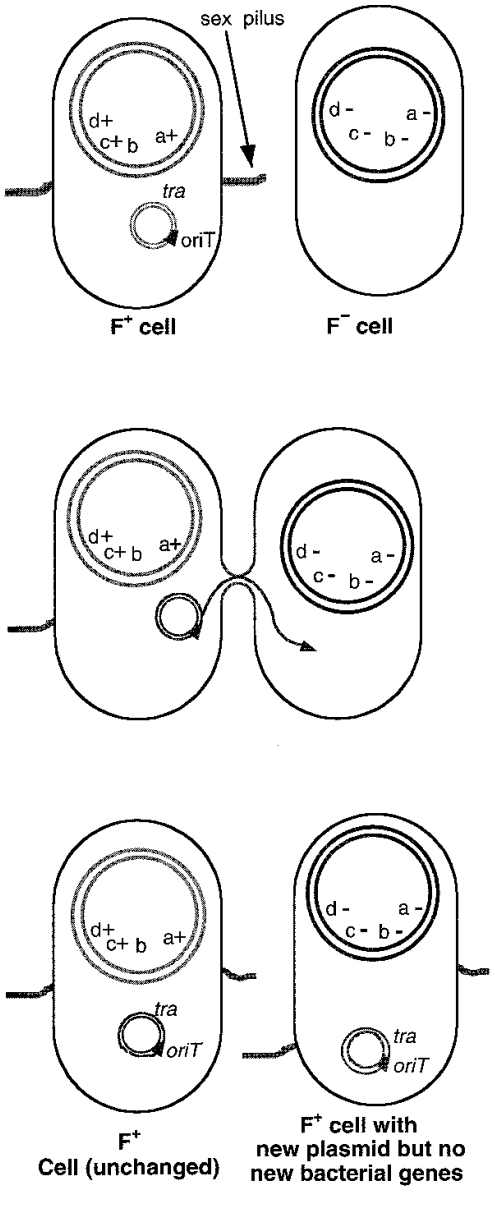
Mating Types



CONJUGAL CROSSES

There are two major types of crosses:

Conjugation: $F^+ \times F^-$ Mating



1. Important points: In the male or F^+ parent, the fertility factor is present but free from the bacterial chromosome. Transfer is unidirectional from male to female. *OriT*, as in every cross, will be transferred first and then the rest of the plasmid genes.

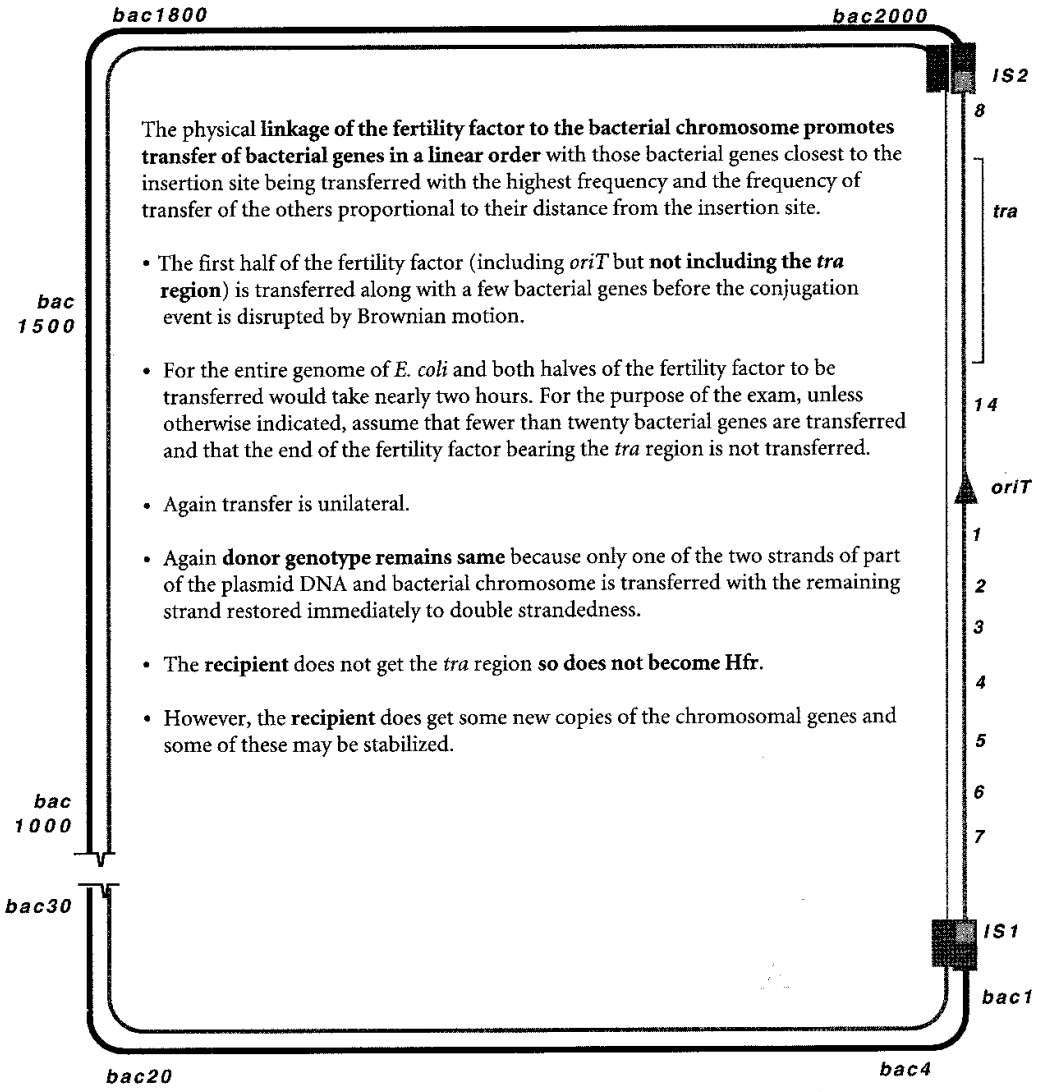
2. Note only a single strand of the plasmid DNA duplex is transferred. The area that is lost is reduplicated (shown as dotted lines) so that the donor always stays the same genotype. The last genes to be transferred are the *tra* region.

3. The transfer of the plasmid is fairly quick so assume that it is transferred in its entirety 100% of the time unless otherwise told. Note that the F^- cell undergoes a sex change becoming F^+ (male). These two F^+ cells can no longer mate. But no BACTERIAL genes are transferred.

Integrated Fertility Factor

Hfr Chromosome
(Bacterial chromosome with integrated F factor)

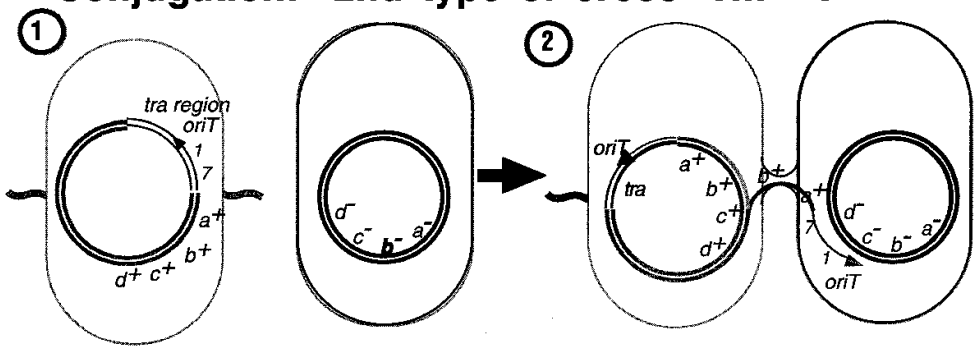
Bacterial genes are represented as ***bac##*** to remind you that there are generally several thousand bacterial genes and that this molecule of DNA is very large.



The physical **linkage of the fertility factor to the bacterial chromosome promotes transfer of bacterial genes in a linear order** with those bacterial genes closest to the insertion site being transferred with the highest frequency and the frequency of transfer of the others proportional to their distance from the insertion site.

- The first half of the fertility factor (including *oriT* but **not including the *tra* region**) is transferred along with a few bacterial genes before the conjugation event is disrupted by Brownian motion.
- For the entire genome of *E. coli* and both halves of the fertility factor to be transferred would take nearly two hours. For the purpose of the exam, unless otherwise indicated, assume that fewer than twenty bacterial genes are transferred and that the end of the fertility factor bearing the *tra* region is not transferred.
- Again transfer is unilateral.
- Again **donor genotype remains same** because only one of the two strands of part of the plasmid DNA and bacterial chromosome is transferred with the remaining strand restored immediately to double strandedness.
- The **recipient** does not get the *tra* region **so does not become Hfr.**
- However, the **recipient** does get some new copies of the chromosomal genes and some of these may be stabilized.

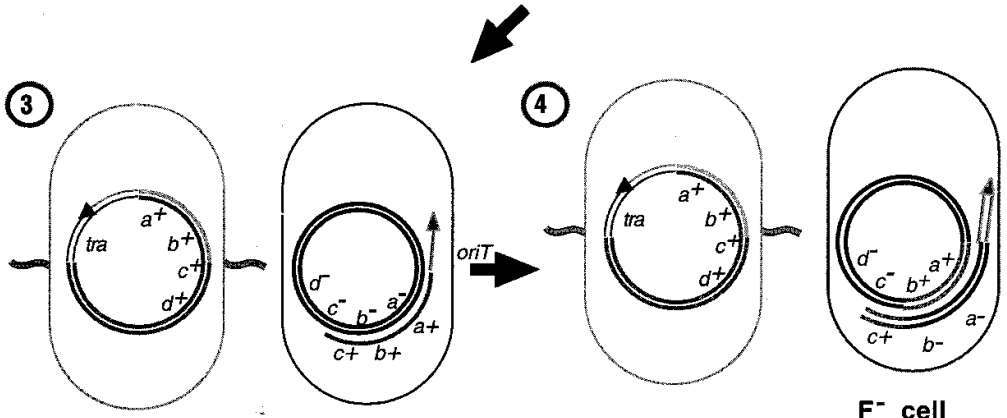
Conjugation: 2nd type of cross Hfr × F⁻



Hfr $a^+b^+c^+d^+$ × $F^-a^-b^-c^-d^-$

Important points: Fertility factor is integrated into the bacterial chromosome. In this cross *oriT* and the first half of the fertility factor (regions 1–7 on the F factor) will be transferred first (and in that order) and then the bacterial genes in the linear order away from the plasmid.

Note, that as with the $F^+ \times F^-$ cross, only a single strand of the DNA duplex is transferred. The area that is lost is reduplicated so that the donor always stays the same genotype. The last genes to be transferred would be the *tra* region.



It takes approximately two hours for a complete transfer to occur. Because the cytoplasmic bridge and DNA strand is so fine, mating is normally interrupted before the transfer is complete. For the purpose of exam, assume that mating is interrupted and the recipient gets some new genes but does not become Hfr.

Hfr cell (unchanged)

F^- cell with new bacterial genes: a^+ and b^+ (No sex change)

Transduction

Transduction is the transfer of bacterial DNA by a phage vector.

The phage picks up the bacterial DNA through an error in phage production.

There are two types of transduction: generalized and specialized.

A generalized transducing phage is produced by the phage putting a piece of bacterial DNA into its head. All genes have an equal chance of being transduced.

- Specialized transduction is dependent on integration of phage DNA into the bacterial chromosome at a specific site and then an error being made in its excision as the phage begins to replicate lytically.

To understand transduction, you need first to understand how a phage replicates normally so that you can understand how the errors are made.

Phage

= bacteriophage = bacterial virus

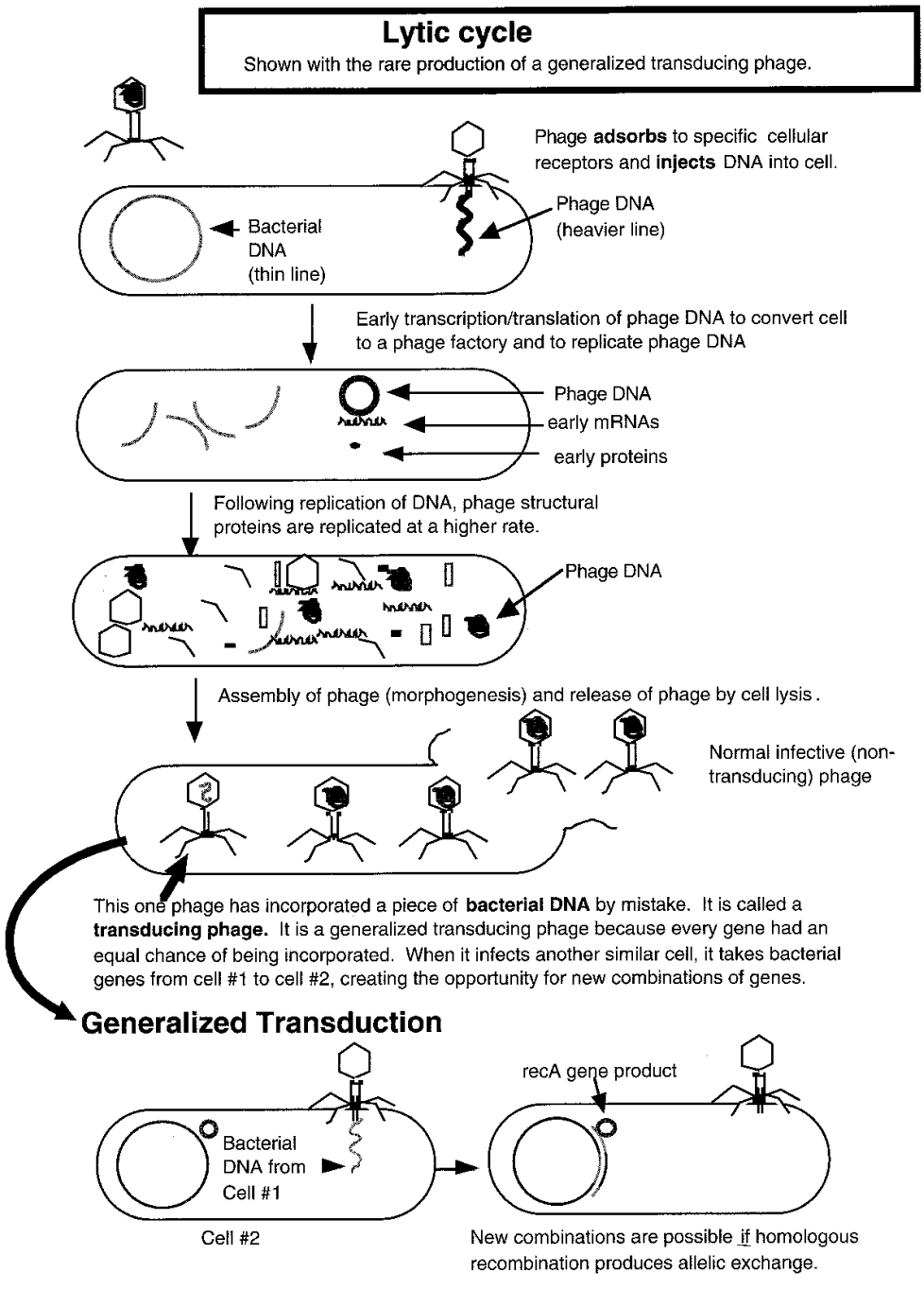
Come in two major types:

- **Virulent phage** infect bacterial cells, always making more virus and lysing the cells (lytic replication).
- **Temperate phage** often infect without lysing the cells because they have the ability to repress active phage replication and to stably integrate their DNA into the bacterial chromosome. In the absence of functional repressor protein, they also may replicate lytically.

Lytic Infection

Lytic infection, by phage or viruses, leads to production of viruses and their release by cell lysis.

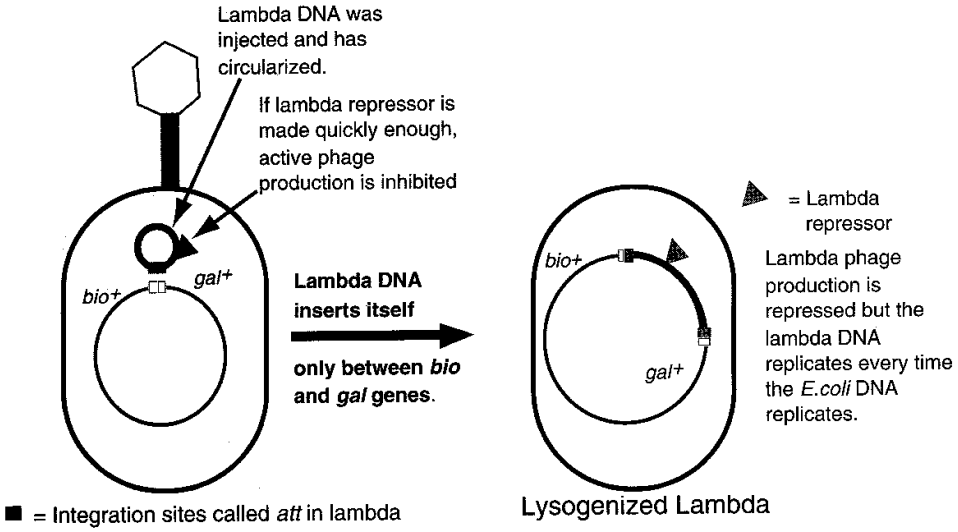
- **Virulent viruses can only go into lytic life and can only carry out generalized transduction.**
- The lytic (or productive) life cycle of virulent phage is shown below. It is entirely normal except for a mistaken incorporation of bacterial DNA into one phage head, creating a transducing virus, shown at the bottom of the next page. Transduction of another bacterial cell is shown following that.


Lytic cycle
Shown with the rare production of a generalized transducing phage.
Phage **adsorbs** to specific cellular receptors and **injects** DNA into cell.
Phage DNA (heavier line)
Bacterial DNA (thin line)
Early transcription/translation of phage DNA to convert cell to a phage factory and to replicate phage DNA
Phage DNA
early mRNAs
early proteins
Following replication of DNA, phage structural proteins are replicated at a higher rate.
Phage DNA
Assembly of phage (morphogenesis) and release of phage by cell lysis.
Normal infective (non-transducing) phage
This one phage has incorporated a piece of **bacterial DNA** by mistake. It is called a **transducing phage.** It is a generalized transducing phage because every gene had an equal chance of being incorporated. When it infects another similar cell, it takes bacterial genes from cell #1 to cell #2, creating the opportunity for new combinations of genes.
Generalized Transduction
recA gene product
Bacterial DNA from Cell #1
Cell #2
New combinations are possible if homologous recombination produces allelic exchange.

Lysogeny and Specialized Transduction

Temperate phage may become prophage (DNA stably integrated) or replicate lytically.

- When repressor is made, temperate phage insert their DNA into the bacterial chromosome where it stably stays as a prophage.
- If the repressor gene gets mutated or the repressor protein gets damaged then the prophage gets excised from the bacterial DNA and is induced into the lytic production of virus. On rare occasions these temperate phage can produce either specialized or generalized transducing viruses. Lambda phage of *E. coli* is the best studied. Most temperate phages have only a single insertion site.
- **Lambda inserts ONLY between *E. coli* genes *gal* and *bio* as shown below.**


Lambda DNA was injected and has circularized.
If lambda repressor is made quickly enough, active phage production is inhibited
bio+
gal+
Lambda DNA inserts itself only between bio and gal genes.
= Lambda repressor
Lambda phage production is repressed but the lambda DNA replicates every time the E.coli DNA replicates.
bio+
gal+
■ = Integration sites called att in lambda
Lysogenized Lambda

Lysogeny

Lysogeny is the state of a bacterial cell with a **stable phage DNA** (generally integrated into the bacterial DNA), **not undergoing lytic replication either because it is repressed or defective.** When the cell DNA replicates, the phage DNA also replicates and, as long as the repressor protein is not damaged, the lysogenic state continues ad infinitum. Defective phage (or defective viruses in the human equivalent) cannot go into an active replication unless a helper virus is present.

Phages that have both options (lytic replication or lysogeny) are called temperate phages. When a temperate phage first infects a cell there is a regulatory race that determines whether the repressor is made fast enough to prevent synthesis of phage components.

The lysogenized cell will replicate to produce two identical cells each with a prophage as long as the repressor gene product is present.

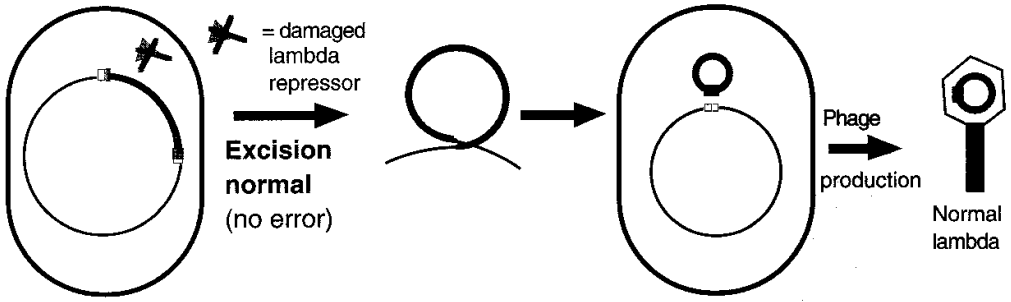
Significance of lysogeny:

- Can confer new properties to a genus such as toxin production or antigens:
  - O: Presence of specific prophage in *Salmonella* can affect **O** antigens.
  - B: Phage CE β or DE β cause *Clost. botulinum* to produce **B**otulinum toxin.
  - E: **E**xotoxins A–C (erythrogenic or pyogenic) of *Strep. pyogenes*
  - D: Prophage beta causes *Corynebacterium diphtheriae* to make **D**iphtheria toxin.
- (Mnemonic for phage-mediated pathogenic factors = OBED)
- Model for retrovirus provirus
- Allows specialized transduction

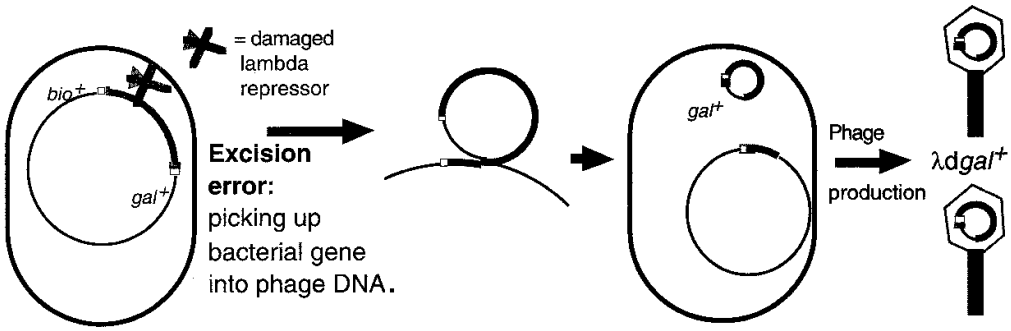
Induction

If the repressor is damaged (by UV, cold, or alkylating agents), then the prophage is excised and the cell goes into lytic replication of the phage. This process is called **induction**.

Most of the time this process is carried out **perfectly as below and the cell produces perfect (non-transducing) normal phage.**



Rarely, in the excision process, an **excisional error** is made and **one of the bacterial genes next to the insertion site is removed attached to the lambda DNA and a little bit of lambda DNA is left behind.**



Because lambda has only one insertion site (between *gal* or *bio*), only *gal* or *bio* can be incorporated by excisional error.

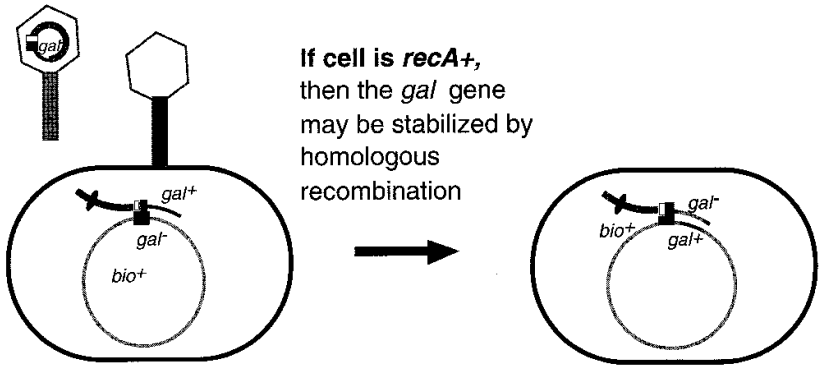
Because all of the phage genes are still in the cell, phage are still made with the circular defective phage genome copied and put in each phage head. These are **specialized transducing phage** (only able to transduce *bio* or *gal*).

Induction with an excisional error is shown above.

Specialized Transduction

Bacterial genes picked up by error in the excision process are transferred to another generally closely related but often genetically distinct cell. If any genes on the exogenote are stabilized by recombinational exchange, then new genetic combinations occur.

Specialized transduction:
λd*gal*$^+$ infects a *gal*$^-$ bacterium



Only those genes next to the phage insertion site can be transduced by specialized transduction.

Table I-6-1. Comparison of Transformation, Conjugation, and Transduction

Requirement	Transformation	Conjugation	Transduction
Is cell-to-cell contact required?	No	Yes	No
Does it require an antecedent phage infection?	No	No	Yes
Is competency required?	Yes	No	No
Is naked (free) DNA involved?	Yes	No	No
Is recombination required to stabilize new genes?	Yes	No for $F^+ \times F^-$ Yes for Hfr $\times F^-$	Yes

Table I-6-2. Comparison of Generalized and Specialized Transduction

	Generalized	Specialized
Mechanism	Error in assembly	Error of excision Requires stable insertion of prophage DNA (lysogeny)
What genes may be transferred?	Any	Only genes next to the insertion site

DRUG RESISTANCE

Overall problem

- Drug resistance is becoming such a significant problem that there are bacteria for which most antibiotics no longer work. Experts have begun to discuss the "post-antibiotic era."
- Drug resistance can be transferred from one genus of bacteria to another, e.g., from your normal flora to a pathogen.
- Three general types of antibiotic resistance: intrinsic, chromosomal-mediated, and plasmid-mediated.

Intrinsic Drug Resistance

For example, a cell which has no mycolic acid will not be inhibited by isoniazid.

Chromosome-Mediated Antibiotic Resistance

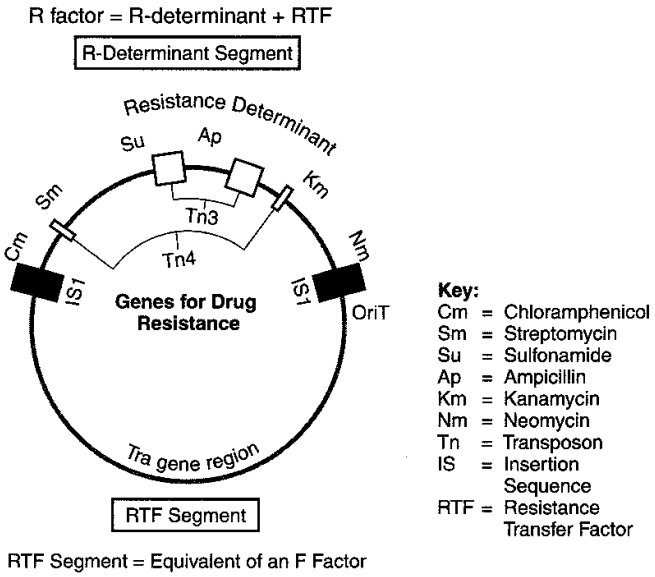
The genes that determine this resistance are located on the bacterial chromosome.

- Most commonly these genes modify the receptor for a drug so that the drug can no longer bind (e.g., a mutation in a gene for a penicillin binding protein [normal cell wall synthetic enzyme]).
- In general, causes low level drug resistance rather than high (exception: methicillin resistance in *Staphylococcus aureus* where a major PBP was mutated).
- But low level resistance may be clinically significant, e.g., in *Streptococcus pneumoniae* meningitis.

Plasmid-Mediated Drug Resistance

The genes that determine this resistance are located on plasmid.

- R factors are conjugative plasmids carrying genes for drug resistance.
  - One section of the DNA (containing *oriT* and the *tra* gene region) mediates conjugation.
  - The other section (R determinant) carries genes for drug resistance. Multiple genes seem to have been inserted through transpositional insertion into a "hot spot."
  - A typical genetic map of an R factor (a conjugative drug-resistant plasmid) is shown on the next page:
- Plasmid-mediated resistance is created by a variety of mechanisms but often genes code for enzymes that modify the drug.



- **Nonconjugative plasmids**
  - Have lost their *tra* operon (genes) **so have lost the ability to DIRECT conjugation.**
  - But as long as they retain their *oriT*, nonconjugative plasmids may actually be transferred by conjugation as long as there is another fertility factor in the same cell with a functional *transfer* region.
  - The process may be referred to as **mobilization** and is able to occur because the genes in the tra region are soluble gene products that are **trans acting. The region *oriT*, by contrast, is cis acting.**

Table I-6-3. Plasmid-Mediated Mechanisms

Antimicrobial Agent	Mechanism
Penicillins and cephalosporins	Production of β-lactamase; cleavage of β-lactam rings
Aminoglycosides	Production of acetyltransferase, adenosyltransferase, or phosphotransferase; inactivation of drug by acetylation, adenosylation, or phosphorylation
Chloramphenicol	Production of acetyltransferase; inactivation of drug by acetylation
Tetracyclines	Increased efflux out of cell
Sulfonamides	Active export out of cell and lowered affinity of enzyme

Transfer of Drug Resistance

Neisseria gonorrhoeae

Nonconjugative plasmids: The segment with the *tra* operon has separated leaving a plasmid with the genes for drug resistance still linked to *oriT*.

Transferred by conjugation (mobilization): As long as the cell still has the *tra* region in the cell, it can direct conjugation, make the nick at *oriT* and mobilize the transfer of the nonconjugative plasmid.

Staphylococcus aureus (Methicillin Resistant = MRSA)

Resistance to methicillin is chromosomal, transferred by transduction. Most of the other antibiotic resistance is transferred by plasmids.

Gram-Negative Bacilli

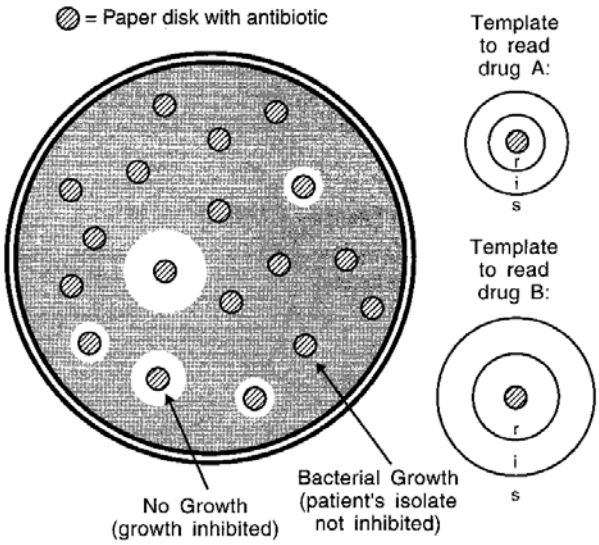
Plasmid mediated, transferred by conjugation.

ANTIBIOTIC SUSCEPTIBILITY TESTING

Kirby Bauer Agar Disk Diffusion Test

- Solid medium with patient's isolate swabbed on the entire plate surface
- Multiple paper disks, each with a single dried drug placed on plate
- Hydration and diffusion of drug set up a concentration gradient during incubation and growth of the bacteria.
- The diameter of the zones of inhibition must be measured to determine significance.
- Only qualitative (reported back as susceptible, intermediate, or resistant)
- Advantages: relatively cheap, easy, can test numerous antibiotics on one plate, wealth of information based on clinical correlation
- Disadvantage: qualitative

Antibiotic Susceptibility Testing
Kirby Bauer Agar Diffusion Plate



"Rapid" Methods

Testing for specific enzymes and a very few probes for genes determining drug resistance are currently available but still require a culture of the patient's pathogen. One current example is β-lactamase testing, shown below.

Patient's bacterial isolate (8–24 hours) + Chromogenic beta lactam —(5–10 min)→ Color change if beta lactamase is present

Minimal Inhibitory Concentration (MIC)

MIC measures antibiotic inhibition.

- This is a dilution technique where **each container** (well of microtiter plate, test tube, or automated system bottle) **has one concentration of an antibiotic** with the patient's isolate. Always one control container has just the patient's isolate and growth medium with no antibiotic to make sure the inoculum is viable.
- **Lowest concentration showing no visible growth is the MIC.**
- In the example below, MIC = 2 μg/ml.
- This indicates levels needed to inhibit; it does not necessarily indicate killing levels, which is done with the MBC (see below).

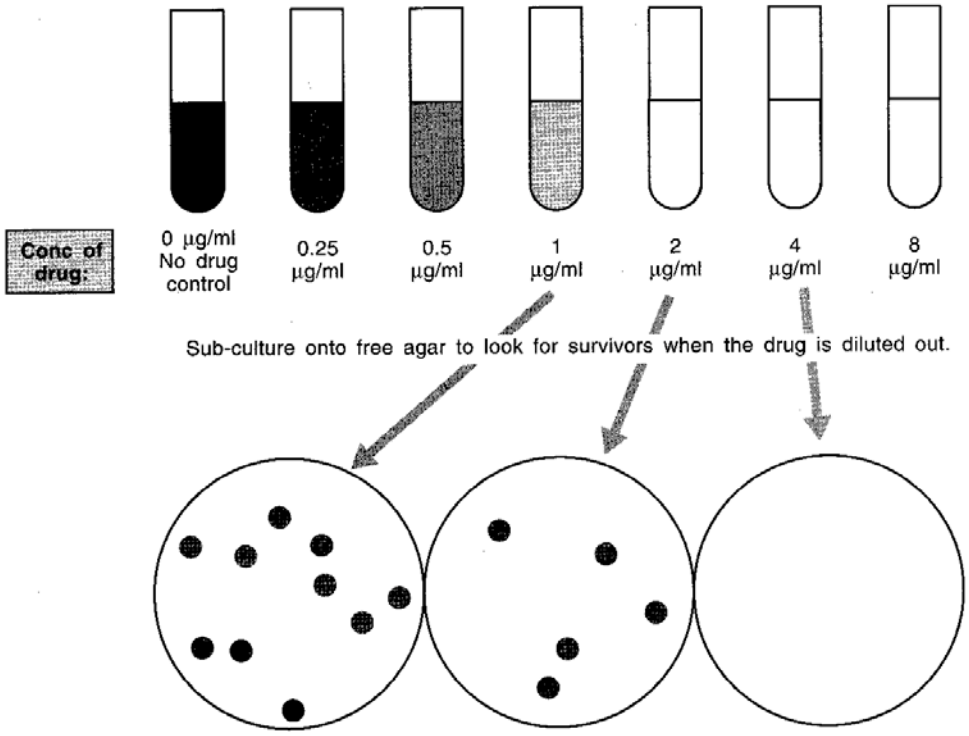
Minimal Bactericidal Concentration (MBC)

This measures the antibiotic killing (bactericidal activity).

- A dilution technique starting with the MIC containers and sub-plating onto solid medium. Because a small inoculum is used on the plate with a large volume of medium, this dilutes the drug way below the MIC and allows determination of viability of cells.
- Important to determine for treating immunocompromised patients whose immune system cannot kill the bacteria while they are inhibited.
- **The MBC is the lowest antibiotic concentration showing no growth on subculture to media without the antibiotic.** In the example below, the MBC would be 4 μg/ml.
- Formulas are increasingly replacing this test.

Minimal Inhibitory Concentration = MICs

1. Each container has one concentration of a drug.
2. Each container has identical inoculum of the patient's bacterial isolate
3. Must run a no drug control.
4. Lowest concentration showing no visible growth = MIC
   (in example, MIC = 2 μg/ml)



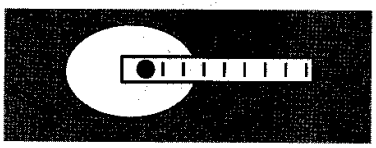
Minimal Bactericidal Concentration (MBC)

(Not routinely done in many hospitals but ordered when necessary)

The lowest drug concentration showing no growth on sub-culture to media without drugs = MBC
MBC in example would be 4 μg/ml.

E-Test (Agar Diffusion)

E-test uses a strip of plastic marked with a gradient unique to each antibiotic that has the dot of dried antibiotic on the underside. This is placed on an agar plate already swabbed with the patient's isolate and read after incubation. It produces a μg/ml value that correlates fairly well with the Minimal Inhibitory Concentration.



Sterilization, Disinfection, Pasteurization

Sterilization: complete removal or killing of all viable organisms.

Disinfection: the removal or killing of disease-causing organisms. Compounds for use on skin: antiseptics.

Pasteurization: the rapid heating and cooling of milk designed to kill milk-borne pathogens such as *Mycobacterium bovis*, *Brucella*, and *Listeria*.

Physical Methods of Control

Heat = saturated steam

- Autoclaving (steam under pressure): 15 lbs pressure → 121°C 15–20 min (sterilizing)
- Dry heat—2 hr 180°C

Radiation

- UV: formation of thymine–thymine pairs on adjacent DNA bases

Filtration

- HEPA (High Efficiency Particulate Air) filters for air
- Nitrocellulose or other known pore-size filters
  - 0.45 μm filters out most bacteria except Mycoplasmas and other cell wall-less forms.
  - 0.22 μm will filter out all bacteria and spores.

Chemical Methods of Control

Agents damaging membrane

- **Detergents:** (surface active compounds) most notable the quaternary ammonium compounds like benzalkonium chloride—interact with membrane through hydrophobic end disrupting membrane.
- **Alcohols:** disrupt membrane and denature protein.
- **Phenols** and derivatives: damage membrane and denature proteins.

Agents modifying proteins

- **Chlorine:** oxidizing agent inactivating sulfhydryl-containing enzymes
- **Iodine and iodophors** (which have reduced toxicity): also oxidation of sulfhydryl-containing enzymes
- **Hydrogen peroxide:** oxidizing agent (sulfhydryl groups); catalase inactivates
- **Heavy metals:** (silver and mercury) bind to sulfhydryl groups inhibiting enzyme activity
- **Ethylene oxide:** alkylating agent (sterilizing agent)
- **Formaldehyde** and glutaraldehyde: denatures protein and nucleic acids and alkylates amino and hydroxyl groups on both

Modification of nucleic acids

- **Dyes:** like crystal violet and malachite green whose positively charged molecule binds to the negatively charged phosphate groups on the nucleic acids

Chapter Summary

The definitions of polymerases, nucleases, and alleles and the flow of genetic information are briefly reviewed.

Three types of DNA may be found in a bacterial cell: chromosomal, plasmid, and bacteriophage DNA.

The chromosomal DNA contains all the essential bacterial genes. Most bacteria have one chromosome but may have multiple copies of it. The chromosomal DNA exists as a large covalently closed circular strand, looped around a proteinaceous center, and contains about 2,000 genes. Each loop can be transcribed independently.

Plasmids are small, covalently closed circular DNAs that replicate autonomously and may be transferred from one bacterium to another. Episomes are a type of plasmid integrated into the bacterial chromosome. Plasmids may code for fertility factors, antibiotic resistance, and exotoxins.

Bacteriophage (viral) DNA may be inserted into the bacterial chromosome as a prophage by a temperate virus. Such an inserted viral gene sometimes directs the synthesis of a virulence factor, making the bacterium more pathogenic.

Transposons are elements of DNA that independently move from one site to another on a plasmid or chromosome. Insertion may cause mutations in unrelated genes and may cause repeats on both sides of the transposon. Transposons may carry genes for drug resistance and play an important role in the development of multiple drug resistance.

Homologous recombination is a process in which linear DNA introduced by transformation, conjugation, or transduction is exchanged for near-homologous DNA on the chromosome. This stabilizes the newly introduced DNA, which as a consequence has now become an integrated part of the bacterial genome.

Site-specific recombination is the integration of a nonhomologous circular DNA molecule into the bacterial genetic material.

Excision is the reversal of site-specific recombination in which an integrated prophage or plasmid may be removed from the bacterial genome.

(Continued)

Chapter Summary (*continued*)

New genetic information is introduced into bacterial cells by gene transfer followed by recombination. The modes of gene transfer are transformation, conjugation, and transduction.

Transformation is the incorporation of naked DNA from the environment. This process is probably of minor biologic import but was used to transform rough Streptococcal cells into smooth ones in the now classic experiments that confirmed the genetic role of DNA.

Conjugation is the direct transfer of DNA from one bacterial cell to another.

Conjugation occurs by direct contact mediated by a fertility (F) plasmid. Donor (male) cells are F^+, and receptor (female) cells are F^-. A strand of the plasmid from the F^+ cell is transferred across the conjugal bridge formed by a sex pilus. The net result is that both cells end up with a complete copy of the plasmid.

A second type of conjugation occurs when a high-frequency recombination (Hfr) donor cell, which has its F factor incorporated into its chromosome, contacts an F^- recipient cell. In this case, the attempt to transfer the whole genome is aborted before completion, and only a portion of the genetic material is transferred and incorporated into the recipient's chromosome.

Transduction is the transfer of bacterial DNA by a bacterial virus (phage). Phages are either virulent (lytic) or temperate (lysogenic). Virulent phages always replicate and lyse their hosts. Temperate phages can repress their lytic behavior and stably integrate their DNA into the bacterial chromosome. If anything interferes with the repressive mechanisms, they revert to a lytic form. Generalized transduction occurs when a lytic phage lyses a bacterium and some bacterial DNA accidentally recombines with phage DNA and is subsequently transferred into another bacterial cell. Specialized transduction occurs when the excision of integrated phage DNA (a prophage) includes some chromosomal DNA. This sometimes occurs when a temperate virus is induced to become lytic by damage to the repressor system or infection with a "helper" virus.

Drug-resistant bacteria have become a major medical problem. Drug resistance arises by three different mechanisms: intrinsic, chromosome-mediated, or plasmid-meditated. Intrinsic resistance is that which is normal for a species. Chromosome-mediated resistance is brought about by mutation, and plasmid-mediated resistance is transferred by conjugation. Transfer of plasmid DNA is the most important way of dispersing drug resistance among bacteria.

Antibiotic susceptibility may be tested by the Kirby-Bauer agar diffusion test, by testing for specific resistance causing enzymes, by the minimal inhibitory concentration test, by the minimal bactericidal test, or by the E (agar diffusion) test.

Pathogenic contamination may be controlled by sterilization or disinfection. Methods of control include heating (e.g., autoclaving, dry heat, or pasteurization); radiation, filtration, or chemicals that destroy the membrane (e.g., detergents, alcohols, or phenols); denaturing proteins (e.g., chlorine, iodine and iodophors, H_2O_2, heavy metals, ethylene oxide, or formaldehyde); or modifying nucleic acids (dyes).

Review Questions

1. What type of genetic material is created by repeated transpositional recombination events?

   A. Chromosomal drug resistance genes
   B. Genetic operon
   C. Hfr chromosome
   D. Insertion sequences
   E. Multiple drug resistance plasmids

2. Which genetic material is found in pathogenic *Corynebacterium diphtheriae* but not in nonpathogenic *C. diphtheriae*?

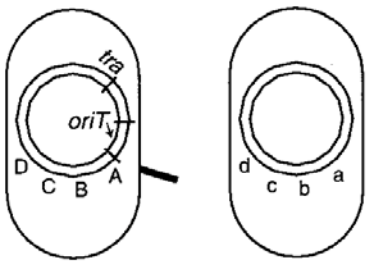
   A. A diphthamide on EF-2
   B. An episome
   C. An F factor
   D. An integrated temperate phage
   E. Highly repetitive bacterial DNA

3. How is a prophage created?

   A. Through activation of the *recA* gene product of an exogenote
   B. Through infection of a bacterial cell with a virulent bacteriophage
   C. Through site-specific recombination of a temperate phage and bacterial DNA
   D. Through infection of a bacterial cell with lambda phage, lacking the lambda repressor
   E. Through excision of bacterial DNA and active lytic replication of a bacteriophage

4. If one cell of type one (figure below) is mixed into a culture of 100 cells of type two (below), and culture conditions are optimized for conjugation BUT NOT for cell division, the cellular genotype that would predominate after overnight incubation would be that of

   A. Cell #1
   B. Cell #1 with new a, b, c, and d alleles
   C. Cell #2 with new A, B, C, and D alleles
   D. Cell #1 with a new a allele
   E. Cell #2 with a new A allele
   F. Cell #1 with new a and b alleles
   G. Cell #1 with new A and B alleles



Cell #1 Cell #2

5. Assume the following cells have no other plasmids other than those mentioned. Which cell type would contain two molecules of DNA?

   A. F^+
   B. F^-
   C. Hfr

6. Assume the cells whose genotype is listed below have no other plasmids than those indicated by the indicated genotype. Which bacterial cell is most likely to transfer chromosomal genes in linear order?

   A. F^+
   B. F^-
   C. Hfr

7. What bacterial gene transfer process is most sensitive to extracellular nucleases?

   A. Conjugation
   B. Generalized transduction
   C. Homologous recombination
   D. Site-specific recombination
   E. Specialized transduction
   F. Transformation

8. Following specialized transduction, if any of the bacterial genes transferred in are to be stabilized, what process must occur?

   A. Conjugation
   B. Generalized transduction
   C. Homologous recombination
   D. Site-specific recombination
   E. Specialized transduction
   F. Transformation

9. The ability of a cell to bind DNA to its surface and import it is required for which genetic process?

   A. Conjugation
   B. Generalized transduction
   C. Homologous recombination
   D. Site-specific recombination
   E. Specialized transduction
   F. Transformation

10. Process by which bacterial or plasmid DNA may be mistakenly incorporated (during assembly) into one phage being produced by the lytic life cycle and then that DNA-transferred to another bacterial cell which may acquire some new genetic traits is called

    A. Conjugation
    B. Generalized transduction
    C. Homologous recombination
    D. Site-specific recombination
    E. Specialized transduction
    F. Transformation

11. Recombination is required for stabilization of genetic material newly transferred by all of the following processes EXCEPT

    A. Movement of a transposon
    B. Integration of a temperate bacteriophage
    C. Transduction of a chromosomal gene
    D. Conjugal transfer of an R factor
    E. Transformation of a chromosomal gene

12. Lysogenic conversion

    A. is a change in pathogenicity due to the presence of a prophage.
    B. is the induction of a prophage to its virulent state.
    C. is the conversion of a virulent phage into a temperate phage.
    D. refers to the incorporation of a prophage into the chromosome.
    E. is the immunity that a prophage confers on a bacterium.

13. Which of the following events is most likely due to bacterial transformation?

    A. A formerly non-toxigenic strain of *C. diphtheriae* becomes toxigenic.
    B. A non-encapsulated strain of *Streptococcus pneumoniae* acquires a gene for capsule formation from an extract of an encapsulated strain.
    C. A strain of *Neisseria gonorrhoeae* starts producing a β-lactamase encoded by a plasmid similar to a plasmid of another Gram-negative strain.
    D. A gene for gentamicin resistance from an *E. coli* chromosome appears in the genome of a virulent bacteriophage that has infected it.

14. Which of the following mechanisms is most likely to be involved in multiple drug resistance transfer from one cell to another?

    A. Specialized transduction of a chromosomal gene for drug resistance
    B. Transformation of chromosomal genes
    C. Transposition
    D. Conjugation with one parent with a free plasmid carrying drug resistance
    E. Conjugation with one parent with chromosomal drug resistance

15. Which of the following agents, if introduced into a growing culture of bacteria, would halt growth but, if then removed, would allow growth to resume?

    A. Antiseptic
    B. Bacteriocide
    C. Bacteriostat
    D. Disinfectant
    E. Sterilizing Agent

Answers

1. **Answer: E.** Transposition or transpositional recombination is a form of site-specific recombination and is largely responsible for the creation of multiple drug resistant plasmids. Chromosomal drug resistance may arise by movement of a plasmid gene to the chromosome, but it is usually just a solitary gene and not a repetitive event. The Hfr chromosome arises through a single site-specific integration of a fertility factor with the bacterial chromosome.

2. **Answer: D.** This question is asking what carries the genetic code (or, more simply, **codes**) for diphtheria toxin, which must be some kind of DNA, which in turn means that the protein EF-2 can be immediately eliminated. The diphthamide on EF-2 is actually the substrate for the ADP-ribosylation done by the diphtheria toxin. Genes expressing the diphtheria toxin originally enter *C. diphtheriae* as part of the DNA of the temperate corynephage. Integration of this temperate phage results in a stable prophage, which directs the production of the diphtheria toxin.

3. **Answer: C.** Site-specific recombination of phage DNA into bacterial cell DNA by the process of lysogeny creates a prophage. The RecA gene product is necessary for homologous recombination with an exogenote but does not create a prophage. A virulent bacteriophage causes lysis of the host cell and not the production of prophage. The lambda phage is a temperate phage, which can cause lysogeny of infected cells, but the lambda repressor is necessary in such cases to prevent the lytic life cycle. **Choice E** might be the pathway a prophage may choose to reinitiate its bacteriophage lytic lifestyle, but it would not be a means to create a prophage.

4. **Answer: E.** This hypothetical condition describes the mixing of one Hfr cell with 100 F^- recipients. Over time, with no cell division occurring, the one Hfr cell would repeatedly conjugate with the F^- cells and transfer one strand of its chromosomal DNA in sequence, beginning with oriT and theoretically ending with the tra genes. The most frequently transferred bacterial genes also have the greatest likelihood of successful recombination; they are those closest to oriT; in this example, the A allele. The entire chromosome is so large that it is virtually never transferred in its entirety and thus, the tra genes would not be transferred. (Even if tra genes were transferred, oriT and tra genes have no homologous regions in the recipient cell chromosome and so would not successfully recombine within.) Thus, the recipient cell acquires only new chromosomal alleles and NOT the whole fertility factor and never changes phenotype to become an Hfr cell. Therefore, any of the answers with cell one (the Hfr parent) as the dominant type would be wrong.

   The genes are transferred in linear order, so choice A will always be transferred more frequently than any of the later genes.

   Therefore, given sufficient time for conjugation, the cell type that would be most numerous is that of the recipient genotype with a newly acquired allele close to oriT. This means

that the best answer is choice F: cell two with a new A gene. The farther from oriT that the allele is, the less likely that it will be successfully transferred. The distractor, choice C, with all four alleles transferred in, is less likely.

5. **Answer: A.** The F^+ cell would contain both the bacterial chromosome and the fertility factor. The other two would just each have the bacterial chromosome (F^-) or the single DNA molecule of the chromosome with the integrated fertility factor.

6. **Answer: C.** Only F^+ and Hfr can donate genes to a recipient or F^- cell. The F^+ cell would transfer only plasmid genes. The Hfr would be the only one likely to transfer chromosomal genes.

7. **Answer: F.** The nucleic acid from the donor cell is not protected from the environment either by a cell or by a phage coat, but is instead naked and therefore subject to nucleases.

8. **Answer: C.** The DNA is transferred in as a linear piece and must be stabilized by homologous recombination.

9. **Answer: F.** The statement fits the definition of competency required for transformation.

10. **Answer: B.** This obviously is transduction, but what are your clues? First, it says "one phage" rather than all the phage in the cell (as for specialized). Then it also said plasmid DNA could be picked up. For specialized transduction, only episomal plasmid DNA (incorporated into the bacterial chromosome near an attachment site) could be picked up.

11. **Answer: D.** Transpositional movement actually involves a type of recombination called transposition that is a form of site-specific recombination. Site-specific recombination is also involved in integration of a temperate bacteriophage. Both transformation and transduction require homologous recombination as would transfer of Hfr DNA by conjugation. But either F factor or R factor DNA circularizes when it enters a new cell and thus is stable without recombination even as circular DNA is not subject to the cellular exonucleases.

12. **Answer: A.** D is a definition of lysogeny but lysogenic conversion is when lysogeny changes the characteristic of the lysogenized organism. In medicine this usually means an increased pathogenicity from the lysogeny.

13. **Answer: B.** A would require phage infection with a temperate corynephage. B (the answer) is most likely to occur through transformation. C is most likely to take place through a conjugal transfer. D might occur by generalized transduction.

14. **Answer: D.** Multiple drug resistance is almost always plasmid-mediated, which rules out A, B, and E. Transposition is just generally within a cell moving a copy of the DNA to another molecule of DNA within the cell.

15. **Answer: C.** This is the classic description of a bacteriostatic agent.

Clinical Infectious Disease

7

These charts are designed for self-study after the organisms have all been reviewed in class. They represent the basics used in clinical scenarios on the USMLE.

Cover the last column on each chart and write the causative agent(s) on paper. Then think about how the organism causes disease. Is there a major virulence factor?

Abbreviations Used

→ means progressing on to

~ means about or approximately

HIV+ = patient with known human immunodeficiency virus infection

Can be used for anyone who is infected but often used for those who are HIV+ but do not have frank AIDS (in other words, CD4+ count >200)

AIDS = acquired immunodeficiency syndrome (CD4+ count <200)

abd = abdominal

CF = cystic fibrosis

CMI = cell-mediated immunity

CGD = chronic granulomatous disease

GU = genitourinary

IC = immunocompromised

Infl'd = inflamed

Infl'n = inflammation

i.v. = intravenous

mo = month(s)

NF = normal flora

occ = occasional

PMNs = polymorphonuclear cells

pt = patient

RBCs = red blood cells

subQ = subcutaneous

If there are multiple causative agents, at the end of the description there may be a # with the abbreviation "CA." This means you should be able to list that number. If it specifically says "species," you should give species.

Table I-7-1. Diseases of Skin, Mucous Membranes, and Underlying Tissues

Type Infection	Case Vignette/Key Clues	Common Causative Agents
Furuncles, carbuncles	Neck, face, axillae, buttocks	*Staphylococcus aureus*
	Inflamed follicles from neck down	*Pseudomonas aeruginosa* (hot tub folliculitis)
Acne vulgaris	Inflammation of follicles and sebaceous glands	*Propionibacterium acnes*
Cutaneous lesions (may be from scratching mosquito bites, cats)	Initially vesicular; skin erosion; honey crusted lesions; catalase negative organism	*Streptococcus pyogenes*
	Initially vesicular but with longer lasting bullae; catalase positive organism	*Staphylococcus aureus*
Red raised butterfly facial rash	Dermal pain, edema, and rapid spread	*Streptococcus pyogenes* (Erysipelas)
Jaw area swelling with pain, sinus tract formation, yellow granules in exudate	Associated with carious teeth, dental extraction or trauma	*Actinomyces israelii* "lumpy jaw" (Actinomycosis)
Hot inflamed tissues	Deeper tissues from extension of skin lesions or wounds including surgical	Variety of bacteria: *S. aureus, S. pyogenes,* Gram - rods, *Clostridia* and anaerobes (cellulitis)
Vesicular lesions	Sometimes preceded by neurologic pain	Herpes
	Sometimes large	*Staphylococcus aureus*
SubQ granulomas/ ulcers/cellulitis	Tropical fish enthusiasts; granulomatous lesion	*Mycobacterium marinum*
	Cellulitis following contact with saltwater or oysters	*Vibrio vulnificus*
	Solitary or lymphocutaneous lesions, rose gardeners or florists, sphagnum moss	*Sporothrix schenckii* (Rose Gardener's disease)
	Subcutaneous swelling (extremities, shoulders), sinus tract formation, granules; multiple CA	Bacteria: *Actinomyces, Nocardia,* Fungi: *Madurella, Pseudallescheria* (Mycetoma)
Malignant pustule	Pustule → dark red fluid-filled, tumor-like lesion → necrosis → black eschar surrounded by red margin	*Bacillus anthracis*
Enlargement from lymphatic blockage	Legs or genitalia with previous painless genital chancre	*Chlamydia trachomatis* L1-3
	Fever, headache, myalgia, inflammation and then lymphadenopathy and elephantiasis of limbs or genitalia	*Wuchereria* and *Brugia;* mosquito spread
Burns, cellulitis	Blue-green pus, grape-like odor	*Pseudomonas aeruginosa*
Wounds	Surgical wounds (clean)	*Staphylococcus aureus*
	Surgical wounds (dirty)—list groups	*S. aureus, Enterobacteriaceae,* anaerobes
	Trauma - list groups	*Clostridium,* Enterobacteriaceae, *Pseudomonas*
	Animal bites	*Pasteurella multocida*
	Cat scratches resulting in lymphadenopathy with stellate granulomas	*Bartonella henselae*
	Shallow puncture wound through tennis shoe sole	*Pseudomonas aeruginosa*
Target lesion, generally with fever, headache	(Not necrotic) rashy border; bite site	*Borrelia burgdorferi*

Table I-7-2. Ear, Nose, Throat, Upper Respiratory System Infections

Type Infection	Case Vignette/Key Clues	Common Causative Agents
Acute otitis media	Red, bulging tympanic membrane, fever 102–103°; pain goes away if drum ruptures or if ear tubes are patent. -5CA	*Streptococcus pneumoniae* *H. influenzae* (often nontypeable, recurs) *Moraxella catarrhalis* RSV Rhinovirus
Otitis externa	Ear pain—list of organisms	Normal flora often involved Often mixed infections: *Staph aureus* (from NF)* *Candida albicans* (from NF) *Proteus* (water organism) *Pseudomonas* (water)
Malignant otitis externa	Severe ear pain in diabetic; life threatening	*Pseudomonas aeruginosa*
Sinusitis	Sinus pain; low-grade fever	As for acute otitis media
Oral cavitary disease	Painful mouth—overgrowth of spirochetes and fusiform bacteria	*Fusobacterium* and treponemes (normal oral spirochetes)
	Sore mouth with thick white coating (painful red base under); increased risk: premature infants, AIDS, IC pts, pts on antibiotics, vitamin C deficiency	*Candida*
Sore throat	Inflamed tonsils/pharynx, which may be purulent and may develop abscesses; cervical lymphadenopathy, fever, ± stomach upset; ± sandpaper rash	*Streptococcus pyogenes* (Group A Strep) Rash indicates presence of erythrogenic exotoxin A
	White papules with red base on posterior palate and pharynx, fever	Coxsackie A
	Throat looking like Strep with severe fatigue, lymphadenopathy, fever ± rash	Epstein-Barr virus (Downey type II cells)
	Low-grade fever with a 1–2 day gradual onset of membranous nasopharyngitis and/or obstructive laryngotracheitis; bull neck from lymphadenopathy; elevated BUN; abnormal ECG; little change in WBC (toxin)	*Corynebacterium diphtheriae* (diphtheria)
Common cold	Rhinitis, sneezing, coughing; list CA with seasonal peaks	Rhinoviruses (Summer–Fall) Coronaviruses (Winter–Spring)

* NF = normal flora.

Table I-7-3. Eye Infections

Type Infection	Case Vignette/Key Clues	Common Causative Agents
Eyelid	Bilateral eye lid swelling, >10% eosinophilia, muscle pain; earlier GI Sx	*Trichinella*
	Stye; 2 CA	*Staphylococcus aureus* *Propionibacterium acnes*
	Unilateral inflammation at bite site often around eye or mouth; travel to Mexico, travel to Central or South America	*Trypanosoma cruzi*
Conjunctivitis neonate	Red itchy eye(s)/pus; onset 2–5 days Red itchy eye(s)/pus; onset 5–10 days Neonate with "sticky eye"	Bacterial pink eye *Neisseria gonorrhoeae* *Chlamydia trachomatis* (serotype D–K U.S.) *Staphylococcus aureus*
Conjunctivitis	Red itchy eye(s), thin exudate; pain, photophobia	Viral pink eye: adenovirus (more common than bacterial pink eye)
	Red eye, pus 3 CA	*S. aureus* Group A Strep, *Strep pneumoniae* (all Gram +) *Haemophilus influenzae* (*H. aegyptius*)
	Red eye, pus, presence of inclusion bodies in scrapings; CA with serotypes in U.S.	*Chlamydia trachomatis* serotypes D–K (inclusion conjunctivitis)
	Granulomas and inturned eye lashes, corneal scarring, blindness; CA with serotypes	*Chlamydia trachomatis* serotypes A, B, Ba, C (trachoma)
Chorioretinitis	Neonate or AIDS; 2 CA	*Toxoplasma*, CMV
Retinopathy with keratitis in baby	Mom i.v. drug abuser	*Treponema pallidum* (congenital syphilis)

Table I-7-4. Cardiac Symptoms

Chills, fever, arthralgia, myalgia, back pain, acutely ill, Janeway lesions; emboli	Developing a heart murmur; i.v. drug user	*Staphyloccoccus aureus*
	Not i.v. drug user	*Staphyloccoccus aureus*
Fever with vague symptoms with insidious onset, fatigue, weakness, weight loss, night sweats, anorexia, myalgias; murmur may have been long present; emboli	Poor oral hygiene or dental work	Viridians streptococci (55% of cases in native hearts)
	Bilary or urinary tract infection GU manipulation in elderly men	*Enterococcus faecalis*
Endocarditis in i.v. drug user		*Staphylococcus aureus* Viridians streptococci *Staph. epidermidis* *Aspergillus* (branching <45°) *Candida* (pseudohyphae) *Pseudomonas*

Table I-7-5. Middle and Lower Respiratory System Infections

Type Infection	Case Vignette/Key Clues	Most Common Causative Agents
Respiratory difficulty or obstruction	Inflamed epiglottis; patient often 2–3 and unvaccinated	*Haemophilus influenzae* (epiglottitis)
	Infant with fever, sharp barking cough, inspiratory stridor, hoarse phonation	Parainfluenza virus (Croup)
Laryngotracheitis laryngotracheobronchitis		Viral etiology
Bronchitis	Wheezy; infant or child ≤5 years	RSV
	>5 years	*Haemophilus influenzae, Mycoplasma pneumoniae, Chlamydia pneumoniae*
	With cough >2 weeks, afebrile; >9	*Bordetella pertussis*
Pneumonia	Poorly nourished, unvaccinated baby/child; giant cell pneumonia with hemorrhagic rash	Measles: malnourishment ↑ risk of pneumonia and blindness
	Adults (including alcoholics) Lobar pneumonia or less commonly, bronchopneumonia	*Streptococcus pneumoniae* (rusty sputum)
	Neutropenic pts, burn patients, CGD, CF	*Pseudomonas*
	Pneumonia teens/young adults; bad hacking cough; initially non-productive cough	*Mycoplasma pneumoniae* (most common cause of pneumonia in school age children)
	Atypical with air conditioning exposure especially >50 yr, heavy smoker, drinker	*Legionella* spp.
	Atypical with bird exposure ± hepatitis	*Chlamydia psittaci*
	Foul smelling sputum, aspiration possible	anaerobes, mixed infection (*Bacteroides Fusobacterium, Peptococcus*)
	Alcoholic, abscess formation, aspiration, facultative anaerobic, Gram-negative bacterium with huge capsule	*Klebsiella pneumoniae* (currant jelly sputum)
	AIDS patients with staccato cough; "ground glass" x-ray; biopsy: honeycomb exudate with silver staining cysts	*Pneumocystis carinii*
Pneumonia with influenza	Primary infection Secondary	Influenza virus pneumonia *Streptococcus pneumoniae*
Acute pneumonia or chronic cough with weight loss, night sweats	Over 55, HIV+, or immigrant from developing country	*Mycobacterium tuberculosis*
	Dusty environment with bird or bat fecal contamination (Missouri chicken farmers)	*Histoplasma capsulatum*
	Desert sand SW U.S.A.	*Coccidioides immitis*
	Rotting contaminated wood	*Blastomyces dermatitidis*

Table I-7-6. Genitourinary Tract Infections

Type Infection	Case Vignette/Key Clues	Most Common Causative Agents
Urethritis	Gram-negative diplococci in PMNs in urethral exudate	*Neisseria gonorrhoeae*
	Culture negative, inclusion bodies	*Chlamydia trachomatis*
	Urease positive, no cell wall	*Ureaplasma urealyticum*
	Flagellated protozoan with corkscrew motility	*Trichomonas vaginalis*
	Frequent and painful urination, hematuria, and fever	(Cystitis) #1 *E. coli*, other Gram-negative enterics, *Pseudomonas*, *Proteus*
	Young, newly sexually active individual; Gram-positive cocci	*Staphylococcus saprophyticus*
	As above with flank pain and prominent fever	(pyelonephritis) *E. coli*, *Staphylococcus*
Cervicitis	Friable, inflamed cervix with mucopurulent discharge; probes or culture to distinguish	*Neisseria gonorrhoeae* (Gram-negative diplococci) *Chlamydia trachomatis* (non-staining obligate intracellular parasite) Herpes simplex (virus)
Vaginal itching, pain, discharge odor	Adherent yellowish discharge, pH >5, fishy amine odor in KOH, clue cells; Gram-negative cells dominate	(Bacterial vaginosis) overgrowth of *Gardnerella vaginalis* and anaerobes
	Vulvovaginitis, pruritis, erythema discharge: consistency of cottage cheese	*Candida* spp.
	Foamy, purulent discharge, many PMNs and motile trophozoites microscopically (corkscrew motility)	*Trichomonas vaginalis*
Genital lesions	Genital warts	Human papilloma virus (most common U.S. STD), *Treponema pallidum*
	Multiple painful vesicular, coalescing, recurring	Herpes
	Nontender ulcer healing spontaneously 2–10 weeks	*Treponema pallidum*
	Non-indurated, painful ulcer, suppurative with adenopathy, slow to heal	*Haemophilus ducreyi*
	Initial papule heals; lymph nodes enlarge and develop fistulas; genital elephantiasis may develop	*Chlamydia trachomatis* L1-3

Diarrhea

Dysentery

- Abdominal cramps, tenesmus, and pus and blood in the stool
- Usually associated with invasive bacterial disease in the colon

Diarrhea

- Refers to profuse watery feces
- Most commonly associated with increased secretion of fluid across the mucosal surfaces of the small intestine in response to a toxin or a viral infection
- No inflammatory cells

Table I-7-7. Diarrhea by Intoxication

Most Common Sources	Common Age Group Infected	Incubation Period	Pathogenesis	Symptoms	Duration of Symptoms	Organism
Ham, potato salad, cream pastries	All	**1–6 hours**	**Heat stable enterotoxin is produced in food contaminated** by food handler with skin lesion; food sits at room temperature	**abd. cramps, vomiting, diarrhea; sweating and headache may occur; no fever**	<24 hours	***Staphylococcus aureus***
Rice	All	<6 hours	**Heat stable toxin causes vomiting**		8–10 hours	***Bacillus cereus:* emetic form**
Meat, vegetables	All	>6 hours	Heat labile toxin causes diarrhea (similar to *E. coli* LT)	Nausea, abd cramps, diarrhea	20–36 hrs	*Bacillus cereus*: diarrheal form

Table I-7-8. Microbial Diarrhea: Organisms Causing Noninflammatory Diarrhea

Most Common Sources	Common Age Group Infected	Incubation Period	Pathogenesis	Symptoms	Duration of Symptoms	Organism
Day care, water, nosocomial, fecal-oral	**Infants and** toddlers, some older	1–3 days (fall, winter, spring)	Microvilli of small intestine blunted; mononuclear infiltrate in lamina propria; disaccharidase activity down; glucose coupled transport normal; lactose intolerance may cause build up and osmotic influx creating watery diarrhea	Noninflammatory watery diarrhea, vomiting, fever, and dehydration	**5–7 days**	**Rotaviruses**
Water, food, fecal-oral	**Older kids and adults**	18–48 hours	Jejunal biopsy shows blunting of microvilli; cytoplasmic vacuolization is seen along with mononuclear infiltrates of tissue; virus appears to decrease brush border enzymes causing malabsorption.	Diarrhea, nausea, and vomiting; fever in some	12–48 hours	**Norwalk virus**
Nosocomial	Young kids, IC	7–8 days	?	Diarrhea, fever, and vomiting	8–12 days	**Adenovirus 40/41**
Beef, poultry, gravies Mexican food	All	8–24 hours	**Enterotoxin**	abd cramps and **watery diarrhea,** rarely fever or vomiting	<24 hours	***Clostridium perfringens***
Water, food, fecal-oral	All ages	9–72 hours	**Toxin stimulates adenylate cyclase and causes increase in cAMP in the small intestine without inflammation or invasion.**	**Profuse watery diarrhea with vomiting; fever may be present** (rice water stools)	3–4 days	***Vibrio cholera***
Raw or **undercooked shellfish** prominent)	Anyone eating raw shellfish	5–92 hours	Self-limited gastroenteritis mimicking cholera; there is a severe, rarer dysentery form, no clear enterotoxin; hemolysins, phospholipase and lysophospholipase; tests for invasiveness are negative.	Explosive watery diarrhea along with headache, abdominal cramps, nausea, vomiting, and fever.	Up to 10 days	***Vibrio parahaemolyticus***
Water, uncooked fruits and vegetables	All ages	12–72 hours	**Heat labile toxin (LT) stimulates adenylate cyclase resulting in efflux of water and ions into the small intestine** stabile toxin stimulates guanylate cyclase	Watery diarrhea with some vomiting and sometimes fever	3–5 days	**Enterotoxigenic *E. coli***
Food, water, fecal-oral	Infants in developing countries	2–6 days	**Adherence to enterocytes through pili causes damage to** adjoining microvilli.	Watery to profusely watery diarrhea	1–3 weeks	**Enteropathogenic *E. coli***
Food, fecal-oral **(hamburger)**	50% <10 yrs., all	3–5 days	**Verotoxin, which is a cytotoxin, causes bloody diarrhea** with no invasion of the organism.	Abdominal cramps, watery diarrhea with blood **(no fever)**	7–10 days	**Enterohemorrhagic *E. coli.***
Water, day care, camping, beavers, dogs, etc.	All, children	5–25 days	Cysts ingested; excyst in the duodenum and jejunum; **multiply and attach to intestinal villi by sucking disk.**	**Loose, pale, greasy diarrhea; mild to severe malabsorption** syndrome	1–2 weeks to years	***Giardia lamblia***
Day care, fecal-oral, animals, **homosexuals**	Children, AIDS patients	2–4 weeks	Sporozoites attach to the epithelial surface of the intestine and replicate.	Mild diarrhea in immunocompetent; severe chronic diarrhea in AIDS	4 days to 3 weeks **in AIDS: indefinite**	***Cryptosporidium parvum***

Table I-7-9. Microbial Diarrhea: Organisms Causing Inflammatory Diarrhea/Dysentery

Most Common Sources*	Common Age Group Infected	Incubation Period	Pathogenesis	Symptoms	Duration of Symptoms	Organism
Poultry, domestic animals, water, unpasteurized milk, day care, fecal-oral	All, especially <1 year and young adults	3–5 days	**Multiply in the small intestine; invades epithelium** resulting in inflammation and RBC and WBC in stools.	Diarrhea, abd pain, malaise enteritis with diarrhea, malaise, fever	1–2 days mild; <1 week normal self-limiting	***Campylobacter jejuni***
Poultry, domestic animals, water, day care, fecal-oral	All, especially infants and kids	8–48 hours	Adsorb to epithelial cells in terminal small intestine; penetrate to lamina propria of ileocecal region causing **PMN response and PG response, which stimulates** cAMP and watery diarrhea.	Diarrhea (occ bloody), abdominal cramps, abd tenderness, fever, and nausea w/occ vomiting osteomyelitis in sickle cell anemia	3–5 days; spontaneous resolution	***Salmonella*** gastroenteritis
Water, day care, no animal reservoirs, fecal-oral	All, esp 6 mo to 10 yr.	1–7 days	*Shigella* colonize the small intestine producing at first an enterotoxin-induced watery diarrhea; ultimately the *shigellae* penetrate the colon mucosa producing **shallow mucosal ulcerations and dysentery; septicemia rare.**	Watery diarrhea at first → lower abdominal cramps, tenesmus and abundant pus and blood in the stools (dysentery)	4–7 days; antibiotics can reduce spread	***Shigella***
Milk, wild **domestic animals** water, fecal-oral	All, esp older kids and young adults	2–7 days	The terminal ileum is infected with enlargement of the mesenteric lymph nodes; produces focal necrosis **difficult to distinguish from appendicitis; organism is able to grow in cold;** produces heat insensitive enterotoxin. Arthritis may occur.	Fever, diarrhea (frequently with leukocytes & blood in stools), abdominal pain; also a noninflam gastroenteritis	1 day – 3 weeks (avg. 9 days)	***Yersinia enterocolitica***
Associated with **antibiotic use**	Pt on antibiotics	NA	Intense inflammatory response creates the friable yellow plaque-like colonic lesions (pseudo-membrane) associated with this disease	Mild diarrhea to severe **colitis;** abdominal cramps; spiking fever, systemic toxicity; blood, mucus, and pus in stools	Until antibiotic stopped; treat with metronidazole	***Clostridium difficile***
Food, water, fecal-oral	Adults	2–3 days	Similar to *Shigella* dysentery	Fever and cramps with blood and pus in the stools	1–2 weeks; fluid and electrolyte replacement	**Enteroinvasive *E. coli***
Food, water, fecal-oral, tropical generally	All	2–4 weeks	Ingested cysts survive (trophozoites die) and multiply in the colon with invasion of the colon wall producing the **characteristic flask-like lesions and extra-intestinal abscesses.**	Gen. acute diarrhea with cramping; sometimes dysentery; ulceration of colon may produce peritonitis	Weeks to months Rx with metronidazole followed by iodoquinol	***Entamoeba histolytica***

Abbreviations: abd = abdominal; esp = especially; occ = occasional.

*Sources: water = those listed are the most common diarrhea diseases spread through water.
Day care = organisms listed are ones which have caused outbreaks in day care facilities, but note that any organism spread by the oral-fecal route may be a problem in this setting.
Milk = unpasteurized milk or dairy products.

Table I-7-10. Other Gastrointestinal or Liver Infections

Signs and Symptoms	Case Vignette/Key Clues	Most Common Causative Agents
Jaundice, anorexia, nausea, right upper quadrant pain on palpation, cigarettes taste foul, elevated liver enzymes*	Food-borne (possibly contaminated raw oysters or clams); 14–45 days; without chronicity; sturdy naked RNA virus	Hepatitis A ("infectious" hepatitis) (picornavirus)
	i.v. drug abuse, needle stick; chronic carrier state, cirrhosis, primary hepatocellular carcinoma; DNA virus easily inactivated by alcohol	Hepatitis B ("serum" hepatitis) neonatal transmission (Hepadnavirus)
	Transfusion or i.v. drug abuse; acute illness is less severe than hepatitis B but chronicity is higher, with 60% of those infected having chronic active hepatitis; RNA, enveloped virus	Hepatitis C (Flavivirus)
	Enterically transmitted with high fatality in pregnant women, no chronic form	Hepatitis E (Calicivirus)
Female with lower abdominal pain; onset often following menses	Adnexal tenderness, bleeding, deep dyspareunia, vaginal discharge, ± fever. Tenderness from cervical movement, possibly palpable inflammatory mass on bimanual exam	*Neisseria gonorrhoeae* or *Chlamydia trachomatis* or both or a variety of other organisms
Acute abdominal pain	Intestinal blockage	*Ascaris lumbricoides* or potentially *Diphyllobothrium latum*
Bile duct blockage following surgery (anesthesia)		*Ascaris lumbricoides*
Peritonitis		Mixed flora often involving anaerobic normal flora: *Bacteroides fragilis* and facultative anaerobes such as *E. coli*
Cirrhosis	Travel history: Puerto Rico, Peace Corps, etc.; egg granulomas block triads → fibrosis	*Schistosoma mansoni*
	i.v. drug use	Hepatitis viruses
Pancreatitis	Generally with swelling of salivary glands	Mumps virus

*Hepatitis may also occur with two other viruses: CMV and yellow fever virus or with toxoplasmosis or leptospirosis.

Table I-7-11. Changes in Blood Cells

Symptoms and Signs	Case Vignette/Key Clues	Most Common Causative Agents
Anemia	Megaloblastic	*Diphyllobothrium latum*
	Normocytic	Chronic infections
	Microcytic and hypochromic (iron deficiency anemia)	*Ancylostoma, Necator, Trichuris*
Patient with cyclic or irregular fever, decreased hemoglobin and hematocrit	Often foreign travel to tropics, schizonts in RBCs	*Plasmodium*
Reduced CD4 cell count		HIV
Increases in PMNs		Generally found in many extracellular bacterial infections
Increases in eosinophils		Allergy
		Helminths during migrations
Increases in mononuclear leukocytes (monocytes or lymphocytes)		Viral intracellular organisms *Listeria, Legionella, Leishmania, Toxoplasma, Pneumocystis*
Increases in lymphocytes (mononucleosis) Fever, fatigue, lymphadenopathy, myalgia, headache	Infectious mononucleosis Heterophile + Downey type II cells (reactive T cells) sore throat, lymphadenopathy, young adult	Epstein-Barr virus (EBV)
	Heterophile negative	CMV *Toxoplasma* *Listeria* (Listeriosis)
Lymphocytosis with hacking cough	Unvaccinated child hypoglycemic	*Bordetella pertussis*

Table I-7-12. Central Nervous System Infections

Signs and Symptoms	Case Vignette/Key Clues	Most Common Causative Agents
Sepsis ± seizures, irritability, or lethargy; rarely bulging fontanelles or nuchal rigidity	Neonate to 2 months	*Streptococcus agalactiae* #1 (Gram-positivecoccus) *E. coli* (Gram-negative rod) More rarely: *Listeria monocytogenes* (motile Gram-positive rod)
Headache, fever, confusion, lethargy, nuchal rigidity, vomiting	6 months to 2 years; no mention of *Haemophilus* vaccine; no indication that child is properly vaccinated	*Haemophilus influenzae* type B* (Gram-negative pleomorphic rod with polyribitol capsule)
	3 mo to young adult prodrome may be very rapid; child may be properly vaccinated; rash	*Neisseria meningitidis* (Gram-negative diplococcus with capsule; ferments maltose)
	<2 yrs Young adults to elderly	*Streptococcus pneumoniae* (Gram-positive coccus, catalase negative, alpha hemolytic, inhibited by optochin, lysed by bile)
	Renal transplant patient	*Listeria monocytogenes* (motile Gram-positive rod)
As above but less toxic and a more gradual onset (several days)		Viral: **Enteroviruses** (~70%): Coxsackie B, echovirus; poliovirus, Coxsackie A. Summer and fall but sporadically all year **Mumps virus** (now rare with vaccine) winter and spring most cases imported from **Lymphocytic choriomeningitis** (exposure to rodents, e.g., hamsters); most cases imported from S. America **Herpes simplex type 2 or Varicella-Zoster**
Several month prodrome (except in severely compromised) with signs of meningitis	Usually some underlying condition	Fungal, e.g., Cryptococcal, or if in Southwestern U.S.: *Coccidioides* If near U.S. great river beds with exposure to bird, bat feces: *Histoplasma capsulatum*
Prefrontal headache, high fever, disturbance of smell	Swimming and often diving in very warm polluted waters	*Naegleria*
Bell's palsy	Systemic disease	*Borrelia burgdorferi*
Guillain-Barré	With GI tract problems	*Campylobacter jejuni*
	With respiratory problems	Influenza

*By 1990, with day care centers and the dramatic increase in *Haemophilus* meningitis, *Haemophilus* meningitis became overall the most common. Since late 1990, when the conjugated vaccine went into use, there has been a dramatic decrease in *Haemophilus* meningitis in **vaccinated kids.**

(*Continued*)

Table I-7-12. Central Nervous System Infections (*continued*)

Signs and Symptoms	Case Vignette/Key Clues	Most Common Causative Agents
Headache, and fever ± drowsiness, coma, hemiplegia, cranial nerve palsy, hallucinations, behavioral disturbances, and other focal neurological findings	Summer-fall, mosquito-borne from bird reservoirs (except for California encephalitis, which is a rodent reservoir)	Encephalitis with **arborviruses:** **Western equine encephalitis** (midwest and west U.S.) **St. Louis encephalitis** elderly most severe infections **California encephalitis** entire U.S. **Eastern equine encephalitis** all age groups but most common in young and old, highest morbidity of viral CNS infections; with mental retardation, seizures, personality changes in survivors
Headache, behavioral changes, lethargy, somnolence → focal deficit (especially frontal temporal lobe involvement)	Focal uptake of radionucleotide, RBCs in CSF	**Herpes simplex encephalitis** (treatable)
Nerve palsies in a patient with tuberculosis and a low CSF glucose	Patient with low CMI	Tuberculous meningitis
Meningoencephalitis in immunocompromised patients		*Acanthamoeba* or *Toxoplasma*
Mass lesion (symptoms dependent on location of mass) and elevated intracranial pressure along with headache, mental changes, nausea, vomiting, fever with chills, and seizure	Generally following: sinus, ear, or dental infection, infection at distant site, head trauma, etc.	Don't do lumbar puncture; CT generally shows ring enhancing lesion; 45% mixed infections; Streptococci and *Bacteroides* are the two most commonly identified groups of bacteria
Child following a viral illness with pernicious vomiting, lethargy and irritability, which may lead to brain swelling	Perhaps indication of aspirin usage, though the linkage is not definitive	Influenza or varicella infection (Reye's syndrome)

Table I-7-13. Cerebrospinal Fluid Finding in Meningitis

Pressure	CSF Appearance	Cell Count (cells/mm^3)	Dominant Cell Type	Glucose mg/dL	Protein mg/dL	Condition
<100 mm H_2O	Clear	0–5	Lymphocytes	40–70	<40	Normal
Normal or +	Clear	0–500	Early: PMNs Late: lymphocytes	Normal or -	Normal or +	Viral infection
++	Opaque	1–60,000	PMNs	-	++	Bacterial infection
+	Clear	10–500	Early: PMNs Late: lymphocytes	-	+ to ++	Fungal infection

– Below normal range, + above normal range

Table I-7-14. Selected Rashes

Type Rash	Progression	Other Symptoms	Disease	Causative Agent/Toxin
Erythematous maculopapular rash (sandpaper-like rash)	Trunk and neck → extremities	Sore throat, fever, nausea	Scarlet fever	*Strep. pyogenes* Exotoxin A-C
Diffuse erythematous, macular sunburn-like rash	Trunk and neck → extremities with desquamation on palms and soles	Acute onset, fever >102°F, myalgia, pharyngitis, vomiting, diarrhea; hypotension leading to multi-organ failure	Toxic shock syndrome	*Staph. aureus* TSST-1
Perioral erythema, bullae, vesicles, desquamation	Trunk and neck → extremities, except tongue and palate; large bullae and vesicles precede defoliation	Abscess or some site of infection	Staphylococcal skin disease: scalded skin disease & scarletina	*Staph. aureus* Exfoliatin
Petechiae → purpura	Trunk → extremities; spares palms, soles, and face	Fever, rash, headache, myalgias, and respiratory symptoms	Epidemic typhus	*Rickettsia prowazekii* ? endotoxin
Petechiae → purpura	Ankles and wrists → generalized with palms and soles	Fever, rash, headache, myalgias, and respiratory symptoms	Rocky Mountain spotted fever (most common on East Coast)	*Rickettsia rickettsii* ? endotoxin
Petechiae → purpura	Generalized (all over)	Abrupt onset, fever, chills, malaise, prostration, exanthem → shock	Early meningococcemia	*N. meningitidis* endotoxin
Skin: maculopapular; mucous membrane: condyloma	Generalized involving the palms and soles	Fever, lymphadenopathy, malaise, sore throat, splenomegaly, headache, arthralgias	Secondary syphilis	*Trep. pallidum* endotoxin
Confluent erythematous maculopapular rash	Head → entire body	Cough coryza, conjunctivitis, and fever (prodrome), oral lesions, exanthem, bronchopneumonia and ear infections	Measles	Rubeola virus Rash from T cell destruction of virus-infected cells in capillaries

Table I-7-15. Osteomyelitis

Type Infection	Case Vignette/Key Clues	Most Common Causative Agents
Fever, bone pain with erythema and swelling, some patients (diabetic particularly, may have associated cellulitis)	Adults, children, and infants without major trauma or special conditions	*Staphylococcus aureus*
	Neonates (<1 mo)	*Staphylococcus aureus* Group B *Streptococcus* Gram-negative rods (*E. coli, Klebsiella, Proteus, Pseudomonas*)
	Sickle Cell Anemia*	*Salmonella*
	Trauma	*Pseudomonas*

* Sickle cell anemia patients are functionally asplenic and may have defective opsonic and alternate complement pathway activities. The most common bacterial infections include
- Encapsulated organisms
  Streptococcus pneumoniae
  Haemophilus influenza type b
  Neisseria meningitidis
  Salmonella enteritidis
- Osteomyelitis due to *Salmonella* sp
- Pneumonia, bacteremia, and meningitis are all a problem.

Table I-7-16. Arthritis Related to Infections

Type Infection	Case Vignette/Key Clues	Most Common Causative Agents
Pain, redness, low-grade fever, tenderness, swelling, reduced joint mobility	#1 overall except in the 15–40 age group where gonococcal is more prevalent	*Staphylococcus aureus*
	Multiple joints	From septicemia, e.g., staphylococci, gonococcal
	15–40 years; mono- or polyarticular	Gonococcal arthritis
	Prosthetic joint	Coagulase negative staphylococci
	Viral	Rubella and hepatitis B parvovirus
	Chronic onset, monoarticular	*M. tuberculosis* or fungal
	Large joint resembling Reiter's following tick bite or erythema migrans	*Borrelia burgdorferi*
Postinfectious (Reiter's)	Following gastrointestinal infection	*Salmonella, Shigella, Campylobacter*, or *Yersinia enterocolitica*

Chapter Summary

This is a self-study chapter consisting of 16 tables organized by organ system. Each table describes the major infectious disease associated with that organ. The information presented has particular relevance to the USMLE. Following these tables are 23 (A–Z) USMLE-style clinical vignettes with open-ended questions and answers. (Please note: This section is not reviewed on video.)

CASE HISTORIES

Case A: A 28-year-old known alcoholic man presents with fever and productive cough. He was basically well until 3 days ago when he noticed perspiration, cough, shaking, chills, and headache. His cough has been associated with the production of a yellowish-green sputum, which occasionally was tinged with brownish streaks, but was not foul smelling. A Gram stain shows Gram-positive cocci in pairs and short chains.

A. What laboratory tests could you use to identify the genus?

Answer: Catalase test (negative) to genus.

B. When plated on blood agar, what other bacterium might you isolate and confuse the causative agent with, and why? What test(s) could distinguish the two?

Answer: Viridans Strep; optochin and bile.

C. What procedure would you perform to type the isolate?

Answer: Quellung reaction with known antibodies to capsule (not antibodies to cell-wall antigens).

Case B: The patient in Case A developed meningitis and died.

A. What would be the expected CSF cell count?

Answer: High.

B. What would be the expected CSF protein and sugar values?

Answer: Protein high, sugar low.

Case C: A 60-year-old male presents with ulceration of the fingers and sensory loss.

A. What is the status of his CMI?

Answer: Leonine facial appearance and the multitude of lesions is strongly suggestive of lepromatous leprosy, so CMI is depressed and lepromin test is negative.

B. Does the organism grow intracellularly or extracellularly?

Answer: Intracellularly-obligate pathogen.

Case D: A patient presents with multiple, crusted and oozing, **honey-colored lesions.**

A. What is the skin infection?

Answer: Impetigo.

B. What two bacteria would you expect to isolate on culture?

Answer: Strep pyogenes (often honey-colored crusted) and/or Staph aureus (often longer-lasting vesicular or with bullae).

C. How would you separate the two?

Answer: Catalase test positive for Staphylococcus, *negative for* Streptococcus

Case E: A patient had intermittent bouts of general malaise, fever with weight loss, and progressive anemia. She presents also with a heart murmur.

A. What additional physical sign might occur and what causes it?

Answer: Splinter hemorrhages as seen in subacute bacterial endocarditis (also seen in trichinosis and sometimes without either of these diseases).

B. What is her underlying condition and the most commonly involved bacteria?

Answer: Damaged heart valve; Viridans streptococci (associated with bad oral hygiene or dental work) or Enterococcus faecalis or E. faecium if she has had bowel surgery.

C. How would you distinguish the colony on blood agar?

Answer: Alpha hemolytic not inhibited by optochin; a viridans streptococcus.

Case F: A mother brings her 2-year-old son to the emergency room because of fever and a stiff neck. Examination reveals an acutely ill child with a temperature of 104°F. CSF is Gram stained, examined in a rapid test, and also cultured. A Gram stain shows pleomorphic, gram-negative rods.

A. In an unvaccinated child, what laboratory test could confirm the identity of the isolate?

Answer: Meningitis screen, a series of immunologic rapid identification tests (usually EIAs using known antibodies), followed by growth of CSF sediment or filtrate on special media and drug susceptibilities.

B. What growth factors are required to grow the isolate on blood agar?

Answer: X = hemin and V = NAD.

Chocolate agar provides both X and V.

C. What is the drug of choice?

Answer: Cefotaxime or Ceftriaxone.

D. What part of his routine health care is he likely missing?

Answer: His Haemophilus vaccination and perhaps others.

Case G: A 23-year-old woman presents with lower back pain, fever, and dysuria of 3 days' duration. Urinalysis reveals many white blood cells (WBC) and WBC casts. Gram stain of the uncentrifuged urine reveals numerous Gram-negative bacilli per oil immersion field. On culture, extremely motile bacteria form waves of confluent growth.

A. What is the most important biochemical characteristic of this organism? Why?

Answer: Urease producing Enterobacteriaceae; kidney stones induced.

Case H: A child developed a unilateral mucopurulent conjunctivitis 10 days after birth. A conjunctival specimen was sent to the laboratory and inoculated into tissue culture cells. Iodine-staining inclusion bodies were produced.

A. What is unusual about the chemical makeup of the organism?

Answer: ATP defective mutant, also muramic acid missing from peptidoglycan.

B. What are the two forms of the organism?

Answer: Elementary bodies (extracellular) and reticulate bodies = replicating forms.

C. What would you see on Gram stain?

Answer: Nothing in the cells—poorly Gram staining.

D. What serologic type caused the child's problem?

Answer: If U.S. kid, serotype D-K.

Case I: A 27-year-old attorney is hospitalized. He was in excellent health until two days earlier when he noted malaise, fatigability, and profound anorexia. He remembers approximately 6–8 weeks ago receiving a tattoo while vacationing in the Caribbean.

A. How would you confirm your clinical diagnosis?

Answer: HBsAg and IgM to HbcAg.

B. What is meant by the "window"?

Answer: A time period between the end of the detectable presence of HBsAg and the beginning of the production of antibody to HbsAg.

HBc antibody and HBeAg are present.

C. What antigen's presence in the past 6 months is indicative that the patient is entering a carrier state?

Answer: HBsAg past 6 months.

D. What antigen correlates with viral production?

Answer: HbeAg.

E. Does the virus carry a virion associated polymerase? If so, what kind?

Answer: Yes, RNA-dependent DNA polymerase (Hepatitis B replicates through an RNA intermediate.)

Case J: A young man became ill with a sore throat and swollen tonsils, marked fatigue, cervical adenopathy, a palpable spleen, and a pruritic erythematous rash that started after self-administration of ampicillin.

A. What is the most likely disease? What are the most common laboratory diagnostic tests? What does the antibody test measure?

Answer: Infectious mononucleosis; monospot test (measures heterophile antibody which is not specific to EBV antigen) plus CBC.

B. What type of cells are the Downey type II cells?

Answer: T lymphocytes. (Reactive cells not infected.)

C. What cells does the virus infect? Through what receptor does the lymphocytic infection begin?

Answer: EBV infects epithelia cells and B lymphocytes, whose receptor is CD21 = CR2.

Case K: A 35-year-old woman presents with a unilateral vesicular rash.

A. The most likely diagnosis is

Answer: Shingles.

B. Describe the virion's nucleic acid.

Answer: Linear dsDNA.

C. Patient had a previous history of what other disease?

Answer: Chickenpox.

Case L: A 27-year-old man presents to the hospital emergency room with a cough, chest pain, and fever. Two days before admission he developed a nonproductive cough. Rales are heard. Gram stain of sputum was negative. Sputa cultures on blood agar were also negative. Culture on a special medium containing cholesterol, purines, and pyrimidines produced colonies in 10 days. Serology 3 weeks later (when he returned because of persistent cough but feeling better) showed cold agglutinins.

A. What is the probable causative agent?

Answer: Mycoplasma pneumoniae.

B. Why did the organism not show up on the Gram stain?

Answer: Organism does not have a cell wall and does not stain with either the primary or counterstain in the Gram stain.

C. What antibiotics do you NOT use?

Answer: Penicillin/cephalosporin.

Case M: A 27-year-old woman presents to the hospital with a fever of 104°F, dyspnea, and cough. Approximately three days ago she noted a papular rash on the face and trunk. At the time of admission there were many skin lesions: some papular, some vesicular, and some umbilicate. She also has similar lesions in her mouth, and her exanthem is shown.

A. The most likely agent causing this condition is:

Answer: Varicella-Zoster.

B. What two viral coded enzymes made both by VZV and HSV make acyclovir a useful drug in treating severe active VZV infections?

Answer: Thymidine kinase and herpes coded DNA polymerase.

Case N: A markedly sick 3-year-old boy presents to the family physician because of fever, dyspnea, cough, and photophobia. In addition, he has a maculopapular eruption on the face and trunk. On examination, it is observed that he has pinpoint gray-white areas with a red base on his buccal mucosae.

A. What is the most probable disease and causative agent?

Answer: Measles; rubeola.

B. What type of vaccine is available and could have prevented these symptoms?

Answer: Attenuated, single serotype of rubeola.

C. To what viral family does the agent belong?

Answer: Paramyxoviruses.

D. Does the virion carry a polymerase? Why?

Answer: Yes, because it is a negative RNA virus.

Case O: A 35-year-old worker at a plant nursery seeks his physician for a suppurative lesion on one of his fingers. A smear is taken of the drainage and stained. Cigar-shaped yeasts are detected.

A. What is the causative agent?

Answer: Sporothrix schenckii.

B. Is the fungus dimorphic or monomorphic?

Answer: This is DIMORPHIC FUNGUS consistent with Sporothrix. You can tell from cigar-shaped yeast (in tissues generally tough to visualize) and hyphae with sleeve and rosettes arrangement of conidia in culture.

C. Treatment

Answer: Itraconazole but oral KI in milk given will also clear up.

Case P: A 24-year-old female returned to the United States after spending six months in Mexico. Ten days ago she started to have attacks of diarrhea and developed abdominal distention. After lunch on the 10th day she noticed marked abdominal discomfort. While the pain was initially mid-abdominal, by 10:00 p.m. it became located predominantly in the right-lower quadrant. She went to the local emergency room where on physical examination it was noted that she had rebound tenderness and a fever of 37.9°C. She became nauseous and vomited.

Laboratory studies revealed a white blood cell count of 20,000/mm^3 with a pronounced eosinophilia. The emergency room physician diagnosed the problem as acute appendicitis. An appendectomy was performed. A smear was made of the appendiceal exudate and knobby oval to barrel-shaped structures measuring 35 × 55 μm were observed.

A. What is the most likely cause of her appendicitis?

Answer: Ascaris lumbricoides migrating into the appendiceal orifice.

B. What major group does the organism belong to?

Answer: Nematodes

Case Q: A 50-year-old Missouri farmer was referred to the hospital because of malaise, weakness, weight loss, fever, and a palpable spleen. Examination of the mouth reveals a painless ulcerated lesion. A punch biopsy of the lesion is obtained and submitted for laboratory study. Histologic study revealed oval structures measuring 2–5 μm, packing the macrophages.

A. What is the most likely causative agent, and what are the distinctive forms?

Answer: Histoplasma capsulatum with the intracellular oval yeasts and the tuberculate macroconidia (and microconidia) in the hyphal state.

B. Where in nature will you find the fungus in large numbers?

Answer: Histoplasma capsulatum: Great central riverbed plains. Chicken coops in Missouri 100% infected. Indianapolis has had an ongoing outbreak and has major problems with it disseminating in their AIDS patients; NY City also high.

Case R: A 64-year-old male is hospitalized because of dementia. One month prior to admission he complained of headaches. On physical examination it is noted that he has nuchal rigidity, disorientation to time and place, and marked confusion. Lumbar puncture reveals 100 WBCs, which are predominantly lymphocytes, protein 85 mg/dl and sugar 45 mg/dl (concomitant blood sugar is 90 mg/dL).

Despite attempts to treat, three weeks after admission he died. An autopsy was performed. Brain sections were stained with H&E. Encapsulated, oval yeasts were revealed in the infected tissue.

A. What is your diagnosis, and why?

Answer: Cryptococcal meningitis; capsule present on yeast in tissues.

B. Who usually acquires the infection?

Answer: Immunosuppressed for meningitis; pulmonary generally only in pigeon breeders or people exposed to extremely high doses.

C. What tests on CSF should have been run?

Answer: The India ink test is very insensitive and cannot rule out Cryptococcal meningitis. Latex particle agglutination is much more sensitive. India ink is still frequently on the exam.

Case S: A 34-year-old accountant presents to the emergency room because of headache and fever of 3 days' duration. The day before admission his wife noted mild confusion and irritability. Lumbar puncture revealed an opening pressure of 300 mm, 200 red blood cells, 90% of the WBCs which are lymphocytes, sugar of 85 mg/dL (concomitant blood sugar of 110 mg/dL), and protein of 65 mg/dL. Bacteriologic smears (and ultimately also the bacterial cultures) were negative, as were India ink preparations. All latex particle agglutination tests for fungal and bacterial capsules done on the patient's CSF were also negative. The patient's condition did not improve despite appropriate therapy, and he died 10 days after hospitalization.

A. What is the most likely diagnosis?

Answer: Herpes simplex encephalitis

B. Does the virus have an envelope?

Answer: Icosahedral with envelope.

C. Where within the cell does the virus replicate?

Answer: Nucleus for both DNA synthesis and assembly.

D. What other members belong to the same family?

Answers: EBV, Varicella-Zoster, Cytomegalovirus.

Case T: A markedly dehydrated patient presents with diarrhea. His stool culture grew organisms only when grown on an alkaline medium. The isolate was oxidase-positive.

A. What is the mechanism of the produced enterotoxin?

Answer: This is Vibrio cholerae: *ADP ribosylation → activating adenylate cyclase → increased cAMP (leaves Gs locked in the active state).*

B. How would you describe his stool specimen?

Answer: Rice water.

Case U: A patient presents with anogenital warts.

A. What is the virus that probably caused the tumors?

Answer: Human papilloma virus.

B. What serotypes are most commonly associated with this clinical presentation?

Answer: 6 and 11

C. Are they premalignant?

Answer: Rarely.

D. What serotypes are most commonly associated with cervical intraepithelial neoplasia?

Answer: 16, 18, and 31. These are sexually transmitted.

Case V: A girl received a bone marrow transplant for the treatment of leukemia. Nine weeks after the transplant her temperature rose, she became dyspneic, and died. Impression smears were taken from the cut surface of the lower lobe of the left lung. The smears were stained with H&E. Intranuclear inclusions with perinuclear clearing were found.

A. Why did the patient develop the pneumonia?

Answer: Immunocompromised—No T cells.

B. How would you describe what you would see (using only two words)?

Answer: Owl's eyes: cells with prominent basophilic intranuclear inclusion bodies.

C. What is the virion's nucleic acid type? To what viral family does it belong?

Answer: dsDNA; Herpes viruses.

Case W: A young woman developed a feverish illness with painful swelling of her knee, elbow, and wrist joints. She has a sparse rash on the distal parts of her limbs, consisting of small hemorrhagic pustules with an erythematous base. A smear was obtained from the exudate of the exanthem and Gram stained. The stain showed intracellular gram-negative diplococci.

A. What disease does she have?

Answer: Disseminated gonococcal infection.

B. Do pili play a role in the pathogenesis?

Answer: Yes, for attachment to epithelial surfaces—colonizing factor along with outer membrane proteins and antigenic variation.

Comparative Microbiology

MORPHOLOGY/TAXONOMY

Spore-Forming Bacteria (Have Calcium Dipicolinate)

Bacillus
Clostridium

Non-motile Gram-Positive Rods

Corynebacterium diphtheriae
Nocardia
Clostridium perfringens (rest of the pathogenic *Clostridia* are motile)
Bacillus anthracis (most other *Bacillus* species are motile)

Acid Fast Organisms

Mycobacterium
Nocardia (partially acid fast)
Cryptosporidium **oocysts**
Legionella micdadei
Isospora oocysts

Bacteria and Fungi That Characteristically Have Capsules

The "biggies" can be remembered by the mnemonic: Some Killers Have Pretty Nice Capsules!

Streptococcus pneumoniae
Klebsiella pneumoniae
Haemophilus influenzae
Neisseria meningitidis
Cryptococcus neoformans (only encapsulated fungal pathogen)
Pseudomonas aeruginosa—slime producer especially in cystic fibrosis patients' lungs
Bordella pertussis

Other Important Capsule Producers
E. coli meningeal strains have capsule, mostly K_1
Bacillus anthracis—poly D-glutamate capsule
Salmonella typhi—(virulence) capsular antigen
Streptococcus pyogenes when first isolated; non-immunogenic (but anti-phagocytic) hyaluronic acid capsule

Biofilm Producers

Staphylococcus epidermidis (catheter-related infections)

Streptococcus mutans (dental plaque)

Pigment Production

***Pseudomonas aeruginosa*—pyocyanin (blue-green, fluorescein)**

Serratia—red pigment

Staphylococcus aureus—yellow pigment

Photochromagenic and scotochromagenic *Mycobacteria*—Carotenoid pigments (yellow and orange)

Corynebacterium diphtheriae—black to gray

Unique Morphology/Staining

Metachromatic staining—*Corynebacterium*

Lancet-shaped diplococci—*Pneumococci*

Kidney bean-shaped diplococci—*Neisseriae*

Bipolar staining—***Yersinia pestis***

Gulls wings—*Campylobacter*

Table I-8-1. Viral Cytopathogenesis

Inclusion Bodies	Virus
Intracytoplasmic (Negri bodies)	Rabies
Intractyoplasmic acidophilic (Guarnieri)	Poxviruses
Intracytoplasmic and intranuclear (Owl's eye)	Cytomegalovirus
Intranuclear (Cowdry's)	Herpes simplex virus Subacute sclerosing panencephalitis (measles) virus
Syncytia formation	**Virus**
Present	Herpes simplex virus Varicella-zoster Paramyxovirus Respiratory syncytial virus HIV

PHYSIOLOGY

Table I-8-2. Metabolism*

Aerobes	Anaerobes	Microaerophilic
Mycobacterium	***Actinomyces***	***Campylobacter***
Pseudomonas	***Bacteroides***	***Helicobacter***
Bacillus	***Clostridium***	
Nocardia	*Fusobacterium*	
Corynebacterium diphtheria	*Prevotella*	
	Propionibacterium (aerotolerant)	
	Eubacterium	
	Lactobacillus (aerotolerant)	

*Most others are considered facultative anaerobes.

Enzymes

Oxidase

- **All Enterobacteria are oxidase negative.**
- **All *Neisseria* are oxidase positive** (as are most other Gram-negative bacteria).

Urease Positive

- ***Helicobacter***
- All ***Proteus* species** produce urease; this leads to alkaline urine and may be associated with renal calculi.
- *Ureaplasma* (renal calculi)
- *Cryptococcus* (the fungus)
- *Nocardia*

Catalase

$$H_2O_2 \xrightarrow{\text{catalase}} H_2O + 1/2\ O_2$$

***Staphylococci* have catalase, *Streptococci* do not.**

Most anaerobes lack catalase.

Catalase positive organisms are major problems in Chronic Granulomatous Disease (CGD):

- *Staphylococcus aureus* (all staphylococci)
- *Pseudomonas aeruginosa*
- *Candida*
- *Aspergillus*
- Enterobacteriaceae, especially *Klebsiella*

Coagulase Positive

- ***Staph aureus***
- ***Yersinia pestis***

DETERMINANTS OF PATHOGENICITY

Genetics

Genes Encoding Pathogenic Factors Reside on:

- The bacterial **chromosome**

  Choleragen

  Endotoxin

  Shiga toxin

  Mnemonic (Chromosomal Encoded Somethings)
- A **plasmid**

  Most toxins and multiple drug resistance
- A **bacteriophage chromosome** stably integrated into the host DNA as a prophage. Virulence modified by the stable presence of phage DNA in bacterial cell = lysogenic conversion.

Examples:

O = *Salmonella* O antigen

B = Botulinum toxin (phage CEβ and DEβ)

E = Erythrogenic toxin of *Streptococcus pyogenes*

D = Diphtheria toxin (Corynephage β)

Mnemonic: **OBED** (or a little pregnant with phage).

Antigenic Variation

Neisseria gonorrhoeae (pili)

Borrelia recurrentis

Trypanosoma brucei

Table I-8-3. Disease Due to Toxin Production

Bacterium	Disease	Activity of Toxin
Corynebacterium diphtheriae	**Diphtheria**	ADP ribosylation of EF-2 results in inhibition of protein synthesis
Clostridium tetani	**Tetanus**	Binds to ganglioside in synaptic membrane, blocks release of glycine
Clostridium botulinum	**Botulism**	Prevents release of acetylcholine
Vibrio cholerae	**Cholera**	Choleragen stimulates adenylate cyclase
E. coli (ETEC)	**Travelers' diarrhea**	LT stimulates adenylate cyclase
Clostridium difficile	Diarrhea	Toxin A and B inhibit protein synthesis and cause loss of intracellular K^+

EF-2 = eukaryotic elongation factor-2.

Heat Stable Toxins

60°C

- *Staphylococcus aureus* enterotoxin
- ST toxin of *E. coli*
- *Yersinia enterocolitica* toxin

100°C

- Endotoxin

Toxins with ADP-Ribosylating Activity

Table I-8-4. Toxins with A-B ADP-Ribosylating Transferase Activity

Toxin	ADP-Ribosylated Host Protein	Effect on Host Cell
Pseudomonas Exotoxin A Exotoxin S	eEF-2 unknown	Inhibits translocation during protein synthesis
Diphtheria toxin	eEF-2	Inhibits translocation during protein synthesis
E. coli heat-labile toxin (LT)	G-protein (G_S)	Increases cAMP in instestinal epithelium causing diarrhea
Cholera toxin	G-protein (G_S)	Increases cAMP in intestinal epithelium causing diarrhea
Pertussis toxin	G-protein (G_i)	Increases cAMP causing lymphocytosis and increased insulin secretion

A is the ADP-ribosyl transferase.
B binds to cell receptor and translocates the A subunit into the cell.

Table I-8-5. Invasive Factors

Invasive Factor	Function	Bacteria
All capsules	Antiphagocytic	See earlier list with morphology
Slime layer (capsule or glycocalyx)	Antiphagocytic	*Pseudomonas*
M protein	Antiphagocytic	Group A Streptococci
A protein	Antiphagocytic	*Staph. aureus*
Lipoteichoic acid	Attachment to host cells	All Gram-positive bacteria
N. gonorrhoeae pili	Antiphagocytic	*N. gonorrhoeae*

Table I-8-6. Extracellular Enzymes

Enzyme	Function	Bacteria
Hyaluronidase	Hydrolysis of ground substance	Group A Streptococci
Collagenase	Hydrolysis of collagen	*Clostridium perfringens* *Prevotella melaninogenica*
Kinases	Hydrolysis of fibrin	*Streptococcus* *Staphylococcus*
Lecithinase (alpha toxin)	Damage to membrane	*Clostridium perfringens*
Heparinase	May contribute to thrombophlebitis	*Bacteroides fragilis*
IgA Proteases	Colonizing factor	*Neisseria* *Haemophilus* *Strep. pneumoniae*

Ability to Survive and Grow in Host Cell

Obligate Intracellular Parasites

Cannot be cultured on inert media. Virulence is due to the ability to survive and grow intracellularly where the organism is protected from many B-cell host defenses.

- Bacteria
  - All Rickettsiae
  - *Chlamydia trachomatis*
  - *Chlamydia psittaci*
  - *Mycobacterium leprae*
- Viruses
  - All are obligate intracellular parasites.
- Protozoa
  - *Plasmodium*
  - *Toxoplasma gondii*
  - *Babesia*
  - *Leishmania*
  - *Trypanosoma cruzi* (amastigotes in cardiac muscle)
- Fungi
  - None

Obligate Parasites That Are Not Intracellular

(e.g., cannot be cultured on inert media but are found extracellularly in the body)

- *Treponema pallidum*
- *Pneumocystis carinii*

Facultative Intracellular Parasites of Humans

- Bacteria
  - *Francisella tularensis*
  - *Listeria monocytogenes*
  - *Mycobacterium tuberculosis*
  - *Brucella* species
  - Non-tuberculous *mycobacteria*
  - *Salmonella typhi*
  - *Legionella pneumophila*
  - *Yersinia pestis*
  - *Nocardia*
  - *Borrelia burgdorferi*
- Fungi
  - *Histoplasma capsulatum*
- Protozoa
  - *Trypanosoma*

EPIDEMIOLOGY/TRANSMISSION

Bacteria That Have Humans as the Only Known Reservoir

Mycobacterium tuberculosis
M. leprae **(armadillos in Texas)**
Shigella **species**
Salmonella typhi
Rickettsia prowazekii **(epidemic typhus)**
Group A β-hemolytic streptococcus
Neisseria meningitidis and *N. gonorrhoeae*
Corynebacterium diphtheriae
Streptococcus pneumoniae
Treponema pallidum
Chlamydia trachomatis

Zoonotic Organisms

(Diseases of animals transmissible to humans)
Bacillus anthracis
Salmonella species except *S. typhi*
Leptospira
Borrelia
Listeria monocytogenes
Brucella species
Francisella tularensis

Pasteurella multocida (cat bites)
Vibrio parahaemolyticus (from fish)
Vibrio vulnificus (oysters)
Yersinia pestis, Y. enterocolitis, Y. pseudotuberculosis
Campylobacter fetus, C. jejuni
Most Rickettsia
Chlamydia psittaci (birds)
Coxiella

Arthropod Vectors in Human Disease: Insects

- Lice
  - Epidemic or louse-borne typhus (*Pediculus h. humanus*)
  - Epidemic relapsing fever
  - Trench fever
- **True bugs**
  - **Chagas' disease (American trypanosomiasis)—kissing bugs (Reduviidae)**
- Mosquitoes
  - **Malaria (*Anopheles* mosquito)**
  - **Dengue (*Aedes*)**
  - **Mosquito-borne encephalitides: WEE, EEE, VEE, SL**
  - **Yellow Fever (*Aedes*)**
  - Filariasis
- **Sandflies**
  - **Leishmanias**
  - Sandfly fever (viral)
  - Bartonellosis
- Midges
  - Filariasis
- Blackflies
  - Onchocerciasis
- Deerflies and horse flies
  - Loaloasis
  - Tularemia
- **Tsetse flies**
  - **African trypanosomiasis**
- **Fleas**
  - **Plague**
  - Endemic typhus

Arthropod Vectors That Are Not Insects

- **Ticks**
  - **Rocky Mountain spotted fever (*Dermacentor, Amblyomma*)**
  - **Colorado tick fever (*Dermacentor*)**
  - **Lyme disease (*Ixodes*)**
  - *Ehrlichia*
  - **Babesiosis (*Ixodes*)**
  - **Tularemia**
  - **Recurrent fever or tick-borne relapsing fever (*Ornithodoros*, a soft tick)**
- Mites
  - Scrub typhus (*Leptotrombium*) (transovarial transmission in vector)
  - Rickettsialpox

Parasitic Infections Transmitted by Eggs

Enterobius vermicularis (pinworm)
Ascaris lumbricoides (roundworm)
Toxocara canis
Echinococcus granulosus
Taenia solium

Bacterial and Fungal Infections That Are Not Considered Contagious

(i.e., no human-to-human transmission)
Nontuberculous mycobacterial infections, e.g., ***Mycobacterium avium-intracellular***
Non-spore forming anaerobes
Legionella pneumophila
All fungal infections except the dermatophytes

Infections That Cross the Placenta

Toxo
Other (Syphilis)
Rubella
CMV
Herpes and HIV
<5% perinatal hepatitis B could possibly have been acquired by crossing placenta.

- Viruses
  - *Cytomegalovirus*
  - *Rubella*
  - Herpes II (in primary infection)
  - Coxsackie B
  - Polio
  - HIV

- Parasites
  Toxoplasma gondii
- Bacteria
  Treponema pallidum
  Listeria monocytogenes

Spread by Respiratory Droplet

Streptococcus pyogenes (Group A)
Streptococcus pneumoniae
Neisseria meningitidis
Mycobacterium tuberculosis
Bordetella pertussis
Haemophilus influenzae
Corynebacterium diphtheriae
Mycoplasma pneumoniae

Influenza
Rubella
Measles
Chickenpox
Pneumocystis carinii

Spread by Inhalation of Organisms from the Environment

Histoplasma
Coccidioides
Blastomyces
Nontuberculous mycobacteria, e.g., *M. avium-intracellulare* (*MAC*)
Legionella
Chlamydia psittaci
Pseudomonas (also spread by ingestion and contact)
Coxiella burnetti (the only Rickettsia that is stable in the environment)

Spread by Oral/Fecal Route

(Infections may be spread by oral sex.)

Salmonella
Shigella
Campylobacter
Vibrio
Yersinia enterocolitica
Yersinia pseudotuberculosis
Bacillus cereus
Clostridium
Staphylococcus (also other routes commonly)

Enteroviruses
Rotavirus
Norwalk agent
Hepatitis A
Polio virus

Toxoplasma—cat feces
Entamoeba
Giardia
Balantidium

All nematodes of interest
Eichinococcus—dog feces

Contact: (Person-to-Person) Nonsexual

Impetigo (*Strep* and *Staph*)
Staphylococcus
Herpes I
Epstein-Barr (kissing)
Hepatitis B (all body fluids)

Contact: Sexual

Chlamydia	HPV
Neisseria	HIV
Treponema	Herpes II
Trichomonas	CMV

PATHOLOGY

Organisms That Produce Granulomas (Persistent Antigen)

Fran Likes My Pal Bruce And His Blasted Cockerspaniel (in) Blessed Salt Lake City. (Mnemonic by M. Free.)

(ic) = intracellular organism
Francisella (ic)
Listeria (ic)
Mycobacterium (ic)
Treponema pallidum
Brucella (ic)
Actinomyces
Histoplasma (ic)
Blastomyces
Coccidioides
Berylliosis
Schistosoma species, sarcoid
Lymphogranuloma venereum (ic)
Cat scratch fever

Infections Causing Intracerebral Calcifications

Toxoplasma

CMV

Cysticercosis

LABORATORY DIAGNOSIS

Special Stains

- Silver stains
  - Dieterle—*Legionella*
  - Gomori methenamine—*Pneumocystis*, fungi
- Acid fast (Ziehl-Neelsen or Kinyoun)
  - *Mycobacterium, Nocardia* (partially AF), *Legionella micdadei, Cryptosporidium*, and *Isospora*
- India ink—*Cryptococcus* (if negative not a reliable diagnostic method)
- Calcofluor white—fungi
- Giemsa
  - Blood protozoa (*Plasmodium, Babesia, Trypanosoma, Leishmania)*
  - *Histoplasma capsulatum* in RES cells

Name Tests

Tests	Disease
PPD or Tuberculin (Mantoux)	TB
Lepromin	Leprosy
Fungal skin tests	Clinically valuable only to demonstrate exposure or anergy
CAMP test	*Strept agalactiae* carriers
Elek test	Toxin producing *C. diphtheriae* strains
Weil-Felix	Rickettsia (with *Proteus* strain OX antigens)

Unusual Growth Requirements

Haemophilus (most species require one or both)

- **X factor** = protoporphyrin IX, the precursor of **hemin**
- **V factor** = NAD (nicotinamide dinucleotide) or NADP

Mycoplasma

- **Cholesterol**

Salt

- ***Staph aureus*** will grow on high salt media.
- Group D enterococci will grow on 6.5% NaCl.
- ***Vibrio parahaemolyticus*** requires NaCl to grow and grows at 6.5%.

Cysteine requirement for growth

- Four Sisters Ella of the Cysteine Chapel (mnemonic by M. Free)
  Francisella, Legionella, Brucella, and *Pasteurella*

Cultures that must be observed for a long time

- *Mycobacterium tuberculosis* and all non-tuberculous mycobacteria **except rapid** growers
- *Mycoplasma pneumoniae*
- *Brucella* sp
- Systemic fungal pathogens (*Blastomyces, Histoplasma,* and *Coccidioides* **in U.S.)**

TREATMENT/PREVENTION

Treat Prophylactically

- *Neisseria meningitidis* (household and day care contacts—**vaccination also used in** outbreaks)
- *Mycobacterium tuberculosis* with a recent skin test conversion or known household (i.e., significant) exposure; or persons **under 35 with a positive** skin test who have never been treated
- *Haemophilus influenzae* B (unvaccinated household contacts <6 years old) —also vaccinate
- *Neisseria gonorrhoeae* (sexual contacts)
- *Treponema pallidum* (sexual contacts)
- *Yersinia pestis*

Vaccines Available in the U.S.

Inactivated Vaccines

- Pertussis (killed whole cell in DTP)
- *Vibrio cholera*
- Influenza virus
- Salk polio (killed)—all primary vaccinations in U.S., including IC patients
- Rabies (HDC or RVA)
- Japanese encephalitis and several other encephalitis vaccines
- Hepatitis A

Live, Attenuated Vaccines

- *Francisella tularensis*
- Measles (rubeola)
- Rubella
- Mumps (killed vaccine available for IC patients)
- Sabin polio (oral)
- Smallpox
- Yellow fever
- Varicella-Zoster

Live, Pathogenic Virus (in enteric-coated capsules)

- Adenovirus

Toxoid: Chemically Modified Toxin—Vaccines

- Tetanus
- Diphtheria
- Pertussis toxoid (in DTaP)

Recombinant Vaccines or Subunit Vaccine

- Hepatitis B—HBsAg (produced in yeast)
- *Haemophilus*—purified capsular polysaccharide conjugated to protein
- *Neisseria meningitidis*—capsular polysaccharides
- Pneumococcal—capsular polysaccharide (23 serotypes)

Chapter Summary

The microorganisms, listed by their taxonomic classification, are divided into the following morphologic groups: spore-forming bacteria, nonmotile Gram-positive rods, acid-fast organisms, capsulated bacteria and fungi, biofilm producers, pigment producers, and bacteria with unique morphology or staining properties. Table I-8-1 lists viruses with inclusion bodies and those that form syncytia.

The micro-organisms are listed according to the following properties related to their physiology: aerobes, anaerobes, and microaerophiles and oxidase, urease, catalase, and coagulase activity.

The microorganisms are listed according to the following properties related to virulence: genetic coding of pathogenic factors, ability to undergo antigenic variation, ability to cause toxin-induced diseases, production of heat-stable toxins, production of toxins that have ADP-ribosylation activity, production of invasive factors, production of extracellular enzymes, and ability to survive and grow in host cells.

The micro-organisms are listed according to the following properties related to epidemiology: bacteria having only human reservoirs; microorganisms that normally cause disease in animals but also can cause human disease; organisms transmitted to humans by arthropod vectors; parasites transmitted to humans as eggs; parasites and bacteria that cause nontransmissible diseases; organisms that cross the placenta; and organisms spread by respiratory droplets, by inhalation, by the fecal/oral route, and by personal nonsexual or sexual contact.

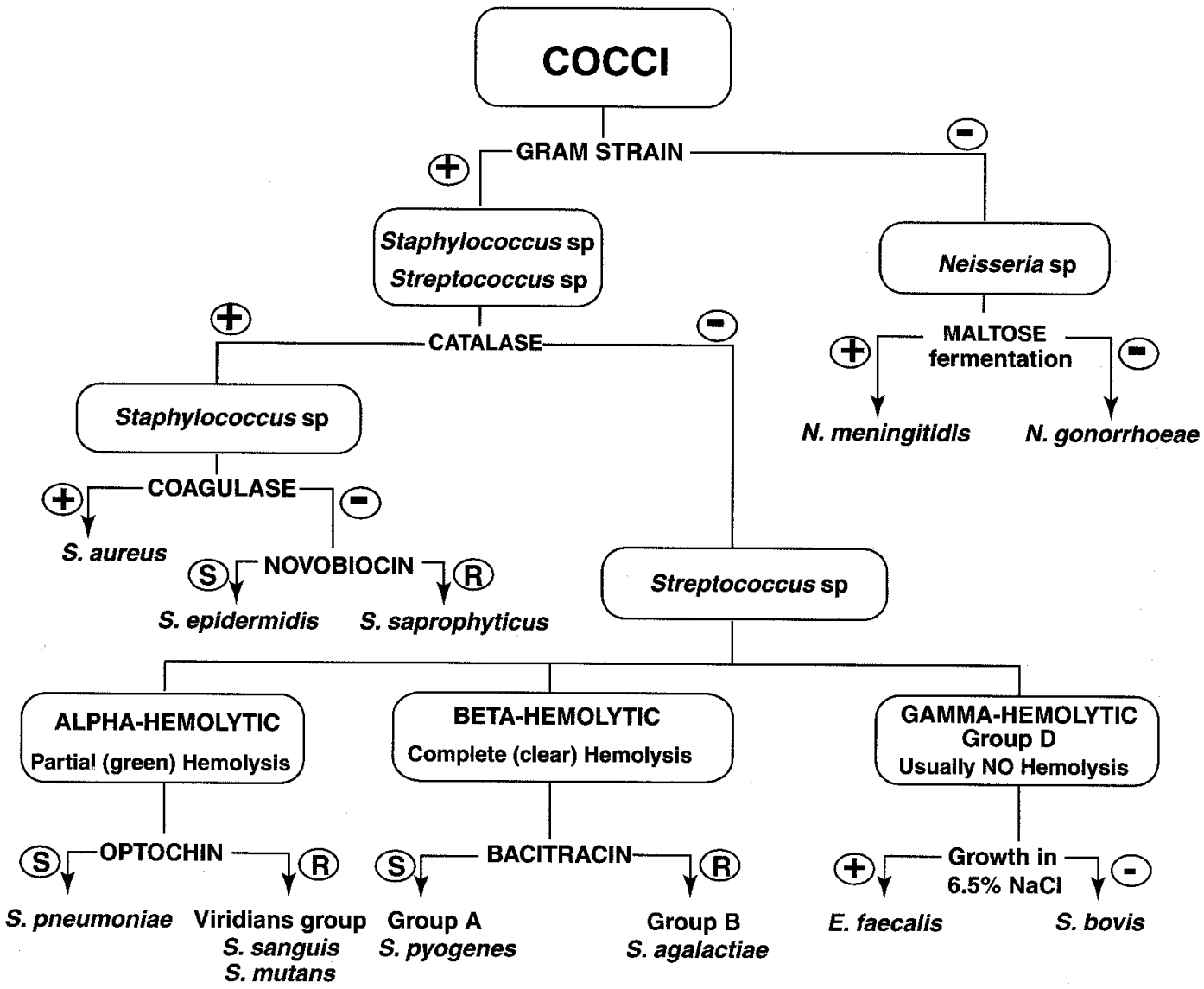
The microorganisms also are listed according to the pathologic production of granulomas and intracerebral calcifications.

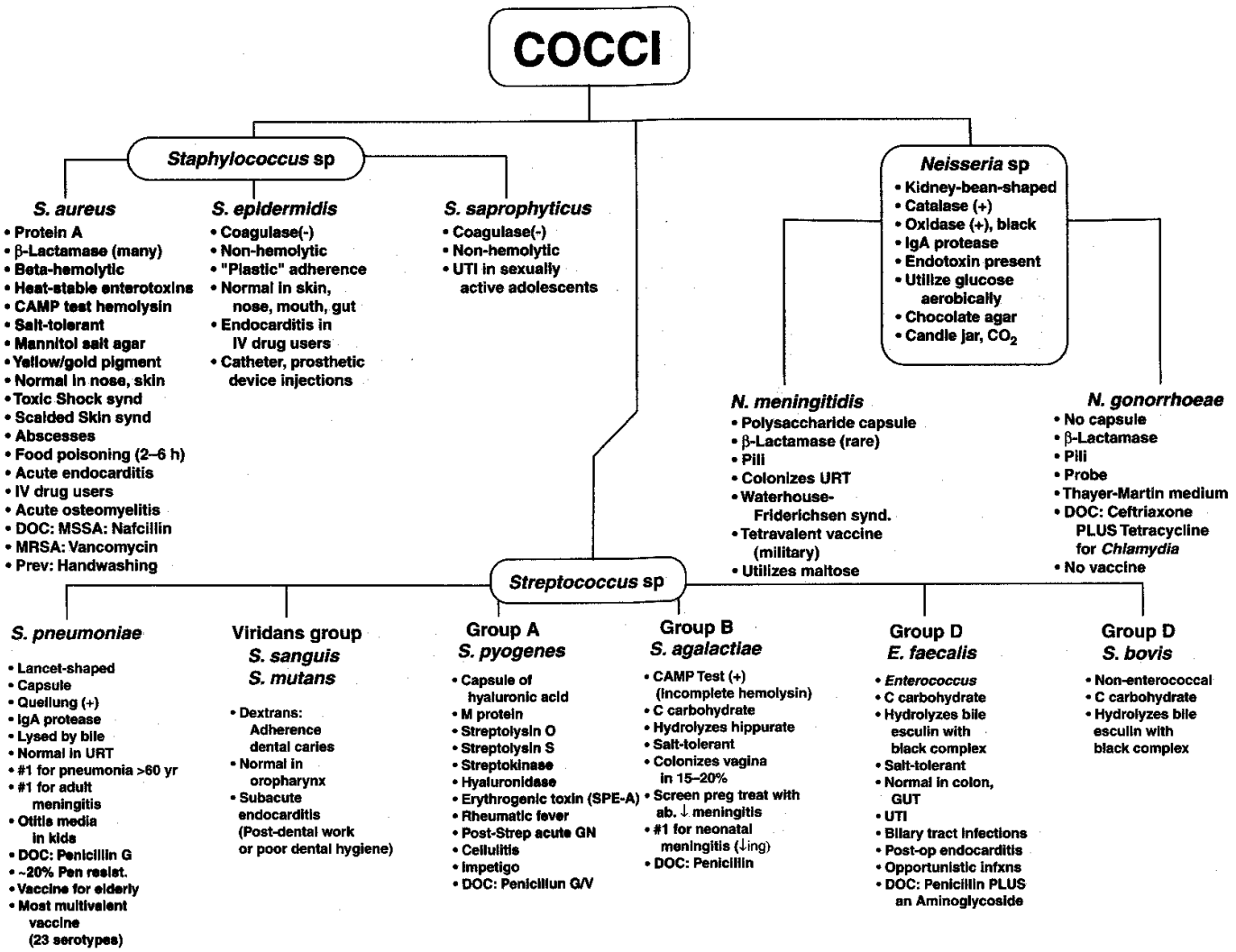
The organisms that can be identified by special stains or by named tests are cataloged.

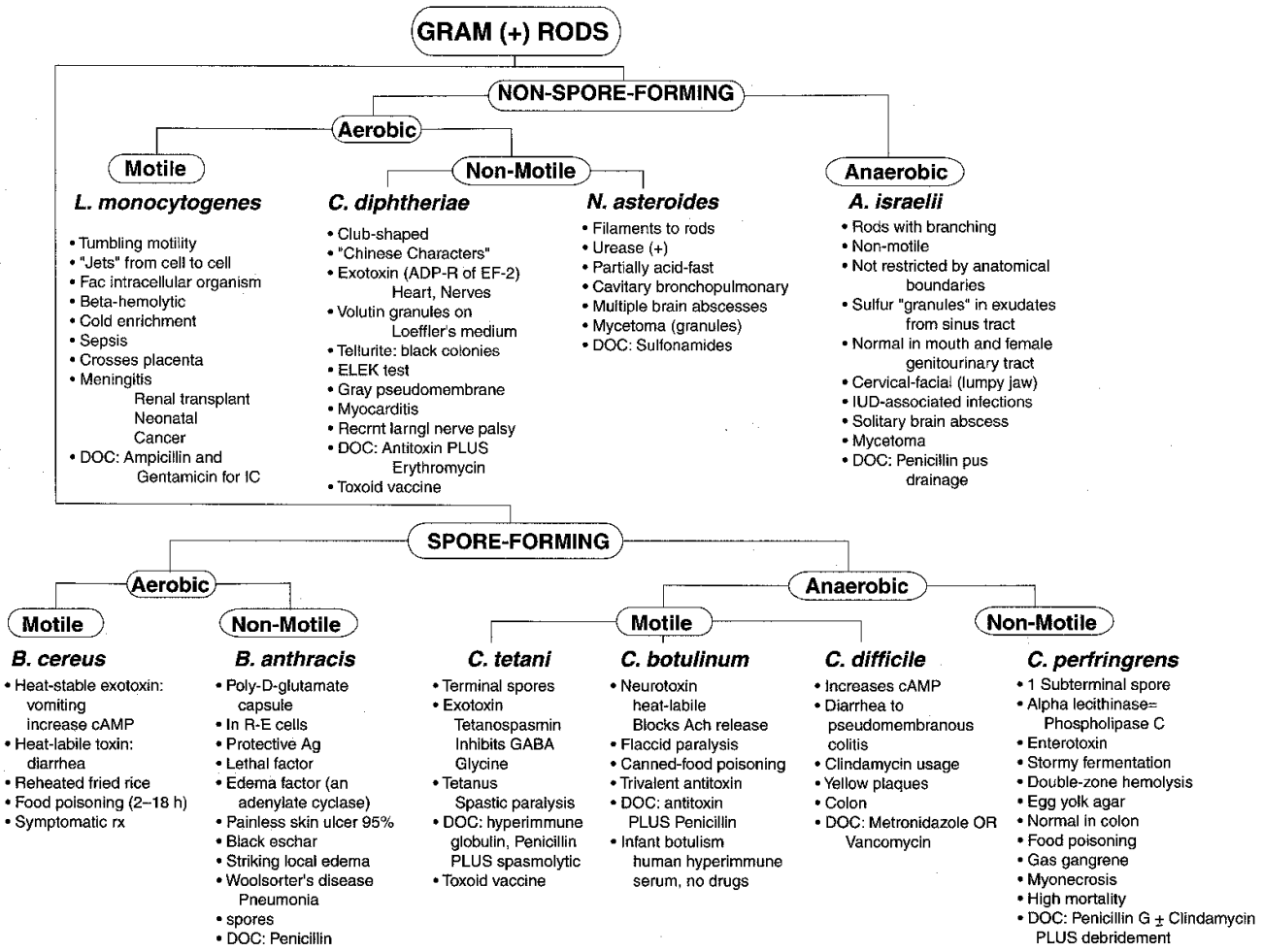
Fastidious organisms and their unusual growth requirements are listed.

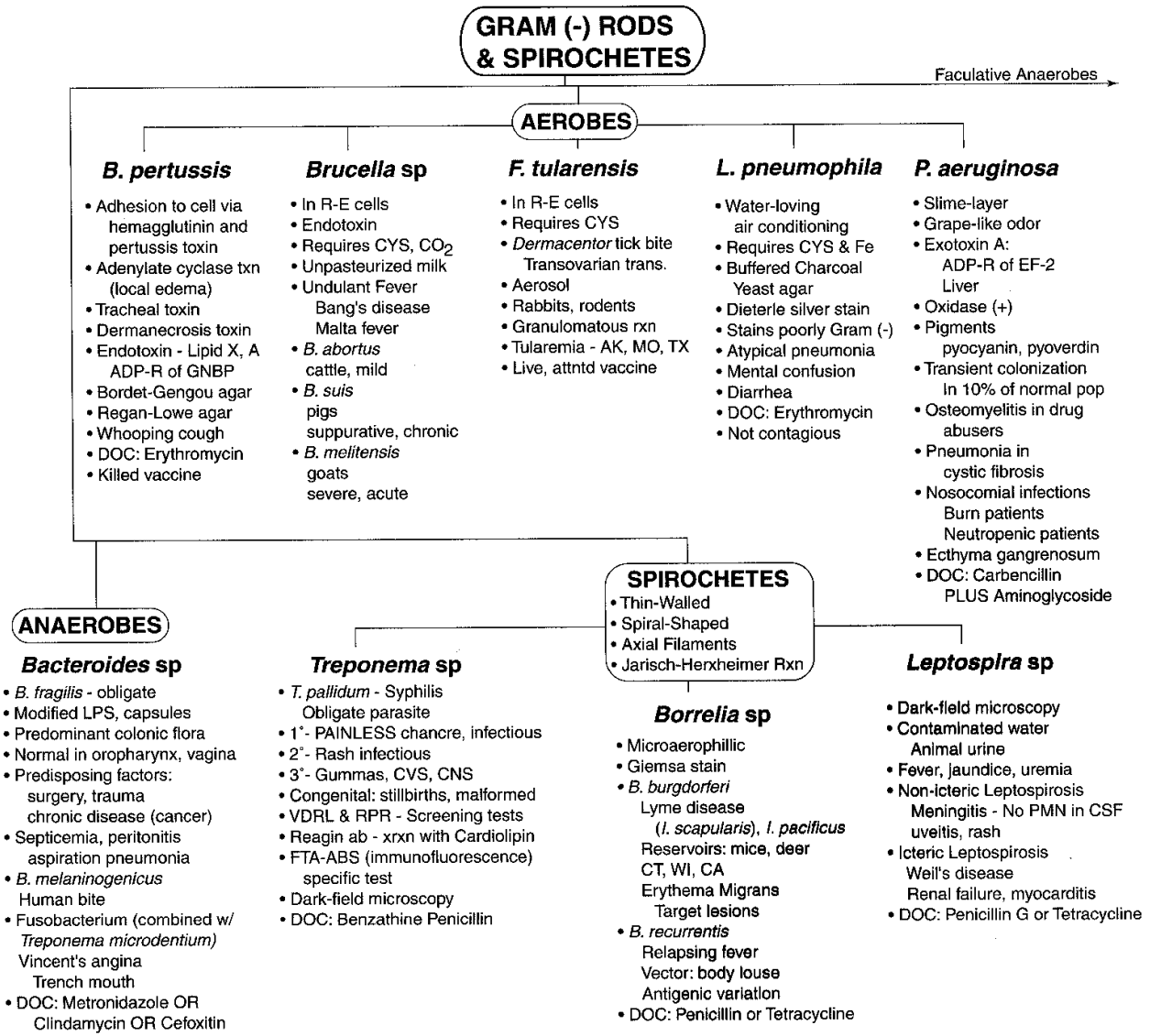
Organisms responding to prophylactic treatments (avoidance, etc.) and for which vaccines are available in the United States are listed. The list includes inactive, live attenuated, live pathogenic, toxoid, and recombinant or component (subunit) vaccines.

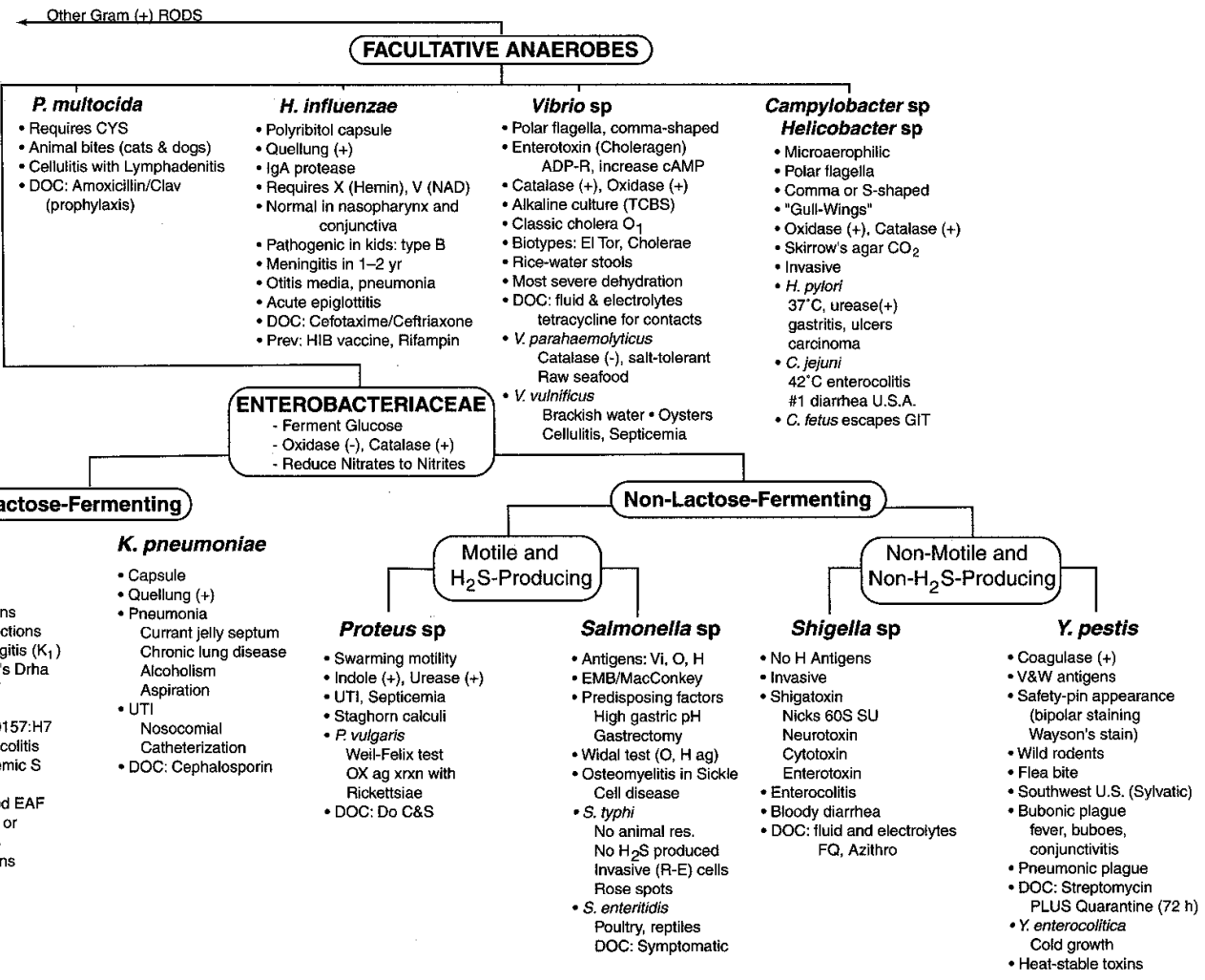
Flow Charts/Clue Sheets


COCCI
GRAM STRAIN
+
-
Staphylococcus sp
Streptococcus sp
Neisseria sp
CATALASE
+
-
MALTOSE fermentation
+
-
N. meningitidis
N. gonorrhoeae
Staphylococcus sp
COAGULASE
+
-
S. aureus
S
NOVOBIOCIN
R
S. epidermidis
S. saprophyticus
Streptococcus sp
ALPHA-HEMOLYTIC
Partial (green) Hemolysis
BETA-HEMOLYTIC
Complete (clear) Hemolysis
GAMMA-HEMOLYTIC
Group D
Usually NO Hemolysis
S
OPTOCHIN
R
S. pneumoniae
Viridians group
S. sanguis
S. mutans
S
BACITRACIN
R
Group A
S. pyogenes
Group B
S. agalactiae
+
Growth in 6.5% NaCl
-
E. faecalis
S. bovis


COCCI
Staphylococcus sp
S. aureus
• Protein A
• β-Lactamase (many)
• Beta-hemolytic
• Heat-stable enterotoxins
• CAMP test hemolysin
• Salt-tolerant
• Mannitol salt agar
• Yellow/gold pigment
• Normal in nose, skin
• Toxic Shock synd
• Scalded Skin synd
• Abscesses
• Food poisoning (2–6 h)
• Acute endocarditis
• IV drug users
• Acute osteomyelitis
• DOC: MSSA: Nafcillin
• MRSA: Vancomycin
• Prev: Handwashing
S. epidermidis
• Coagulase(-)
• Non-hemolytic
• "Plastic" adherence
• Normal in skin, nose, mouth, gut
• Endocarditis in IV drug users
• Catheter, prosthetic device injections
S. saprophyticus
• Coagulase(-)
• Non-hemolytic
• UTI in sexually active adolescents
Neisseria sp
• Kidney-bean-shaped
• Catalase (+)
• Oxidase (+), black
• IgA protease
• Endotoxin present
• Utilize glucose aerobically
• Chocolate agar
• Candle jar, CO_2
N. meningitidis
• Polysaccharide capsule
• β-Lactamase (rare)
• Pili
• Colonizes URT
• Waterhouse-Friderichsen synd.
• Tetravalent vaccine (military)
• Utilizes maltose
N. gonorrhoeae
• No capsule
• β-Lactamase
• Pili
• Probe
• Thayer-Martin medium
• DOC: Ceftriaxone PLUS Tetracycline for Chlamydia
• No vaccine
Streptococcus sp
S. pneumoniae
• Lancet-shaped
• Capsule
• Quellung (+)
• IgA protease
• Lysed by bile
• Normal in URT
• #1 for pneumonia >60 yr
• #1 for adult meningitis
• Otitis media in kids
• DOC: Penicillin G
• ~20% Pen resist.
• Vaccine for elderly
• Most multivalent vaccine (23 serotypes)
Viridans group
S. sanguis
S. mutans
• Dextrans: Adherence dental caries
• Normal in oropharynx
• Subacute endocarditis (Post-dental work or poor dental hygiene)
Group A
S. pyogenes
• Capsule of hyaluronic acid
• M protein
• Streptolysin O
• Streptolysin S
• Streptokinase
• Hyaluronidase
• Erythrogenic toxin (SPE-A)
• Rheumatic fever
• Post-Strep acute GN
• Cellulitis
• Impetigo
• DOC: Penicillin G/V
Group B
S. agalactiae
• CAMP Test (+) (incomplete hemolysin)
• C carbohydrate
• Hydrolyzes hippurate
• Salt-tolerant
• Colonizes vagina in 15–20%
• Screen preg treat with ab. ↓ meningitis
• #1 for neonatal meningitis (↓ing)
• DOC: Penicillin
Group D
E. faecalis
• Enterococcus
• C carbohydrate
• Hydrolyzes bile esculin with black complex
• Salt-tolerant
• Normal in colon, GUT
• UTI
• Biliary tract infections
• Post-op endocarditis
• Opportunistic infxns
• DOC: Penicillin PLUS an Aminoglycoside
Group D
S. bovis
• Non-enterococcal
• C carbohydrate
• Hydrolyzes bile esculin with black complex


GRAM (+) RODS
NON-SPORE-FORMING
Aerobic
Motile
L. monocytogenes
• Tumbling motility
• "Jets" from cell to cell
• Fac intracellular organism
• Beta-hemolytic
• Cold enrichment
• Sepsis
• Crosses placenta
• Meningitis
Renal transplant
Neonatal
Cancer
• DOC: Ampicillin and Gentamicin for IC
Non-Motile
C. diphtheriae
• Club-shaped
• "Chinese Characters"
• Exotoxin (ADP-R of EF-2)
Heart, Nerves
• Volutin granules on Loeffler's medium
• Tellurite: black colonies
• ELEK test
• Gray pseudomembrane
• Myocarditis
• Recrnt larngl nerve palsy
• DOC: Antitoxin PLUS Erythromycin
• Toxoid vaccine
N. asteroides
• Filaments to rods
• Urease (+)
• Partially acid-fast
• Cavitary bronchopulmonary
• Multiple brain abscesses
• Mycetoma (granules)
• DOC: Sulfonamides
Anaerobic
A. israelii
• Rods with branching
• Non-motile
• Not restricted by anatomical boundaries
• Sulfur "granules" in exudates from sinus tract
• Normal in mouth and female genitourinary tract
• Cervical-facial (lumpy jaw)
• IUD-associated infections
• Solitary brain abscess
• Mycetoma
• DOC: Penicillin pus drainage
SPORE-FORMING
Aerobic
Motile
B. cereus
• Heat-stable exotoxin: vomiting increase cAMP
• Heat-labile toxin: diarrhea
• Reheated fried rice
• Food poisoning (2–18 h)
• Symptomatic rx
Non-Motile
B. anthracis
• Poly-D-glutamate capsule
• In R-E cells
• Protective Ag
• Lethal factor
• Edema factor (an adenylate cyclase)
• Painless skin ulcer 95%
• Black eschar
• Striking local edema
• Woolsorter's disease Pneumonia
• spores
• DOC: Penicillin
Anaerobic
Motile
C. tetani
• Terminal spores
• Exotoxin Tetanospasmin Inhibits GABA Glycine
• Tetanus Spastic paralysis
• DOC: hyperimmune globulin, Penicillin PLUS spasmolytic
• Toxoid vaccine
C. botulinum
• Neurotoxin heat-labile Blocks Ach release
• Flaccid paralysis
• Canned-food poisoning
• Trivalent antitoxin
• DOC: antitoxin PLUS Penicillin
• Infant botulism human hyperimmune serum, no drugs
C. difficile
• Increases cAMP
• Diarrhea to pseudomembranous colitis
• Clindamycin usage
• Yellow plaques
• Colon
• DOC: Metronidazole OR Vancomycin
Non-Motile
C. perfringrens
• 1 Subterminal spore
• Alpha lecithinase= Phospholipase C
• Enterotoxin
• Stormy fermentation
• Double-zone hemolysis
• Egg yolk agar
• Normal in colon
• Food poisoning
• Gas gangrene
• Myonecrosis
• High mortality
• DOC: Penicillin G ± Clindamycin PLUS debridement


GRAM (-) RODS & SPIROCHETES
Faculative Anaerobes
AEROBES
B. pertussis
• Adhesion to cell via hemagglutinin and pertussis toxin
• Adenylate cyclase txn (local edema)
• Tracheal toxin
• Dermanecrosis toxin
• Endotoxin - Lipid X, A ADP-R of GNBP
• Bordet-Gengou agar
• Regan-Lowe agar
• Whooping cough
• DOC: Erythromycin
• Killed vaccine
Brucella sp
• In R-E cells
• Endotoxin
• Requires CYS, CO_2
• Unpasteurized milk
• Undulant Fever
Bang's disease
Malta fever
• B. abortus
cattle, mild
• B. suis
pigs
suppurative, chronic
• B. melitensis
goats
severe, acute
F. tularensis
• In R-E cells
• Requires CYS
• Dermacentor tick bite
Transovarian trans.
• Aerosol
• Rabbits, rodents
• Granulomatous rxn
• Tularemia - AK, MO, TX
• Live, attntd vaccine
L. pneumophila
• Water-loving air conditioning
• Requires CYS & Fe
• Buffered Charcoal Yeast agar
• Dieterle silver stain
• Stains poorly Gram (-)
• Atypical pneumonia
• Mental confusion
• Diarrhea
• DOC: Erythromycin
• Not contagious
P. aeruginosa
• Slime-layer
• Grape-like odor
• Exotoxin A:
ADP-R of EF-2
Liver
• Oxidase (+)
• Pigments
pyocyanin, pyoverdin
• Transient colonization
In 10% of normal pop
• Osteomyelitis in drug abusers
• Pneumonia in
cystic fibrosis
• Nosocomial infections
Burn patients
Neutropenic patients
• Ecthyma gangrenosum
• DOC: Carbencillin
PLUS Aminoglycoside
ANAEROBES
Bacteroides sp
• B. fragilis - obligate
• Modified LPS, capsules
• Predominant colonic flora
• Normal in oropharynx, vagina
• Predisposing factors:
surgery, trauma
chronic disease (cancer)
• Septicemia, peritonitis
aspiration pneumonia
• B. melaninogenicus
Human bite
• Fusobacterium (combined w/ Treponema microdentium)
Vincent's angina
Trench mouth
• DOC: Metronidazole OR Clindamycin OR Cefoxitin
SPIROCHETES
• Thin-Walled
• Spiral-Shaped
• Axial Filaments
• Jarisch-Herxheimer Rxn
Treponema sp
• T. pallidum - Syphilis
Obligate parasite
• 1°- PAINLESS chancre, infectious
• 2°- Rash infectious
• 3°- Gummas, CVS, CNS
• Congenital: stillbirths, malformed
• VDRL & RPR - Screening tests
• Reagin ab - xrxn with Cardiolipin
• FTA-ABS (immunofluorescence) specific test
• Dark-field microscopy
• DOC: Benzathine Penicillin
Borrelia sp
• Microaerophillic
• Giemsa stain
• B. burgdorferi
Lyme disease
(I. scapularis), I. pacificus
Reservoirs: mice, deer
CT, WI, CA
Erythema Migrans
Target lesions
• B. recurrentis
Relapsing fever
Vector: body louse
Antigenic variation
• DOC: Penicillin or Tetracycline
Leptospira sp
• Dark-field microscopy
• Contaminated water
Animal urine
• Fever, jaundice, uremia
• Non-Icteric Leptospirosis
Meningitis - No PMN in CSF
uveitis, rash
• Icteric Leptospirosis
Weil's disease
Renal failure, myocarditis
• DOC: Penicillin G or Tetracycline


Other Gram (+) RODS
FACULTATIVE ANAEROBES
P. multocida
• Requires CYS
• Animal bites (cats & dogs)
• Cellulitis with Lymphadenitis
• DOC: Amoxicillin/Clav (prophylaxis)
H. influenzae
• Polyribitol capsule
• Quellung (+)
• IgA protease
• Requires X (Hemin), V (NAD)
• Normal in nasopharynx and conjunctiva
• Pathogenic in kids: type B
• Meningitis in 1–2 yr
• Otitis media, pneumonia
• Acute epiglottitis
• DOC: Cefotaxime/Ceftriaxone
• Prev: HIB vaccine, Rifampin
Vibrio sp
• Polar flagella, comma-shaped
• Enterotoxin (Choleragen) ADP-R, increase cAMP
• Catalase (+), Oxidase (+)
• Alkaline culture (TCBS)
• Classic cholera O1
• Biotypes: El Tor, Cholerae
• Rice-water stools
• Most severe dehydration
• DOC: fluid & electrolytes tetracycline for contacts
• V. parahaemolyticus Catalase (-), salt-tolerant Raw seafood
• V. vulnificus Brackish water • Oysters Cellulitis, Septicemia
Campylobacter sp
Helicobacter sp
• Microaerophilic
• Polar flagella
• Comma or S-shaped
• "Gull-Wings"
• Oxidase (+), Catalase (+)
• Skirrow's agar CO2
• Invasive
• H. pylori 37°C, urease(+) gastritis, ulcers carcinoma
• C. jejuni 42°C enterocolitis #1 diarrhea U.S.A.
• C. fetus escapes GIT
ENTEROBACTERIACEAE
- Ferment Glucose
- Oxidase (-), Catalase (+)
- Reduce Nitrates to Nitrites
Lactose-Fermenting
E. coli
• Normal in colon
• #1 for UTI P-pili, X-Adhesins
• Nosocomial infections
• Neonatal meningitis (K1)
• ETEC • Traveler's Drha Toxins: LT, ST
• EIEC • Invasive
• EHEC • VETC 0157:H7 Hemorrhagic colitis Hemolytic uremic S
• EPEC Plasmid-coded EAF
• DOC: Ampicillin or Sulfonamides Cephalosporins
K. pneumoniae
• Capsule
• Quellung (+)
• Pneumonia Currant jelly septum Chronic lung disease Alcoholism Aspiration
• UTI Nosocomial Catheterization
• DOC: Cephalosporin
Non-Lactose-Fermenting
Motile and H2S-Producing
Proteus sp
• Swarming motility
• Indole (+), Urease (+)
• UTI, Septicemia
• Staghorn calculi
• P. vulgaris Weil-Felix test OX ag xrxn with Rickettsiae
• DOC: Do C&S
Salmonella sp
• Antigens: Vi, O, H
• EMB/MacConkey
• Predisposing factors High gastric pH Gastrectomy
• Widal test (O, H ag)
• Osteomyelitis in Sickle Cell disease
• S. typhi No animal res. No H2S produced Invasive (R-E) cells Rose spots
• S. enteritidis Poultry, reptiles DOC: Symptomatic
Non-Motile and Non-H2S-Producing
Shigella sp
• No H Antigens
• Invasive
• Shigatoxin Nicks 60S SU Neurotoxin Cytotoxin Enterotoxin
• Enterocolitis
• Bloody diarrhea
• DOC: fluid and electrolytes FQ, Azithro
Y. pestis
• Coagulase (+)
• V&W antigens
• Safety-pin appearance (bipolar staining Wayson's stain)
• Wild rodents
• Flea bite
• Southwest U.S. (Sylvatic)
• Bubonic plague fever, buboes, conjunctivitis
• Pneumonic plague
• DOC: Streptomycin PLUS Quarantine (72 h)
• Y. enterocolitica Cold growth
• Heat-stable toxins

Poorly Gram-Staining Organisms*

ACID FAST

Mycobacteria

M. tuberculosis
- Gram (+) wall but doesn't stain due to waxy CW
- Acid fast, obligate aerobe
- Respiratory transmission
- Pathogen, contagious
- Cord factor-trehalose mycolate-inhib. WBC migration mitoch. resp./ oxid. phosphor
- Sulfatides-inhib. phagosome, lysozome fusion
- Niacin (+), catalase (-) at 68°C
- Slow growing
- Drug resistance
- Lowenstein-Jensen medium

M. avium-intracellulare
- Gram (+) wall but doesn't stain due to waxy CW
- Acid fast
- Obligate aerobe
- Soil organism
- Opportunist, non-contagious
- Pulmonary → diss infections CA pts, late AIDS pts

M. leprae
- Obligate intracellular bacterium
- Tuberculoid (CMI damage)
- Lepromatous leprosy (poor CMI)

M. marinum
- Cutaneous lesions (fish tank granuloma)

SOME ATP

Rickettsias

R. rickettsii
- Obligate intracellular bacteria
- Gram-negative envelope but stain poorly
- Rocky MT Spt'd Fever-rash on wrists/ankles → trunk, palms, soles
- Vector: *Dermacentor* tick
- Reservoirs: ticks, wild rodents
- Dx: serol: 4x incr indir Fl. Ab
  DOC: Tetracyclines

R. prowazekii
- Obligate intracellular, bacteria
- Epidemic typhus
- Vector: *Pediculus* louse
- Reservoir: humans, squirrel fleas, flying squirrels

Coxiella burnetti
- Obligate intracellular bacteria
- Q fever-atyp pneumo, hepatitis
- Urine, feces, amnionic fluid, placenta-airborne, resistant to drying
- Reservoir: cats, domestic livestock, high titers in pregnant
- Weil-Felix test negative
- No rash

Bartonella henselae
- Cat scratch fever

NO ATP, mod. peptidoglycan

Chlamydias

C. trachomatis
- Obligate intracellular bacteria
- Gram-negative envelope but stain poorly; lack muramic acid
- Elementary body-transmitted
- Reticulate body-intracellular
- Dx: serology or tissue culture growth confirmed by inclusion bodies (Fl Ab, Giemsa, iodine)

Serotypes D-K
- U.S.-Most common bacterial STD (HPV and HSVZ more common)
- Neonatal/adult inclus. conjunct, neonatal. pneumo; urethritis cervicitis, PID, infertility

Serotypes L1, 2, 3
- Lymphogranuloma venereum
- STD in Africa, Asia, S. America

Serotypes A, B, Ba, C
- Trachoma-follic conjunctivitis → conj. scarring, in-turned eye lashes → corneal scarring
- Leading infectious cause blindness
- DOC: Tetracycline or Erythromycin

C. pneumoniae
- TWAR agent
- Respiratory infections
- Probably very common

C. psittaci
- Atypical pneumonia
- Birds

NO CELL WALL

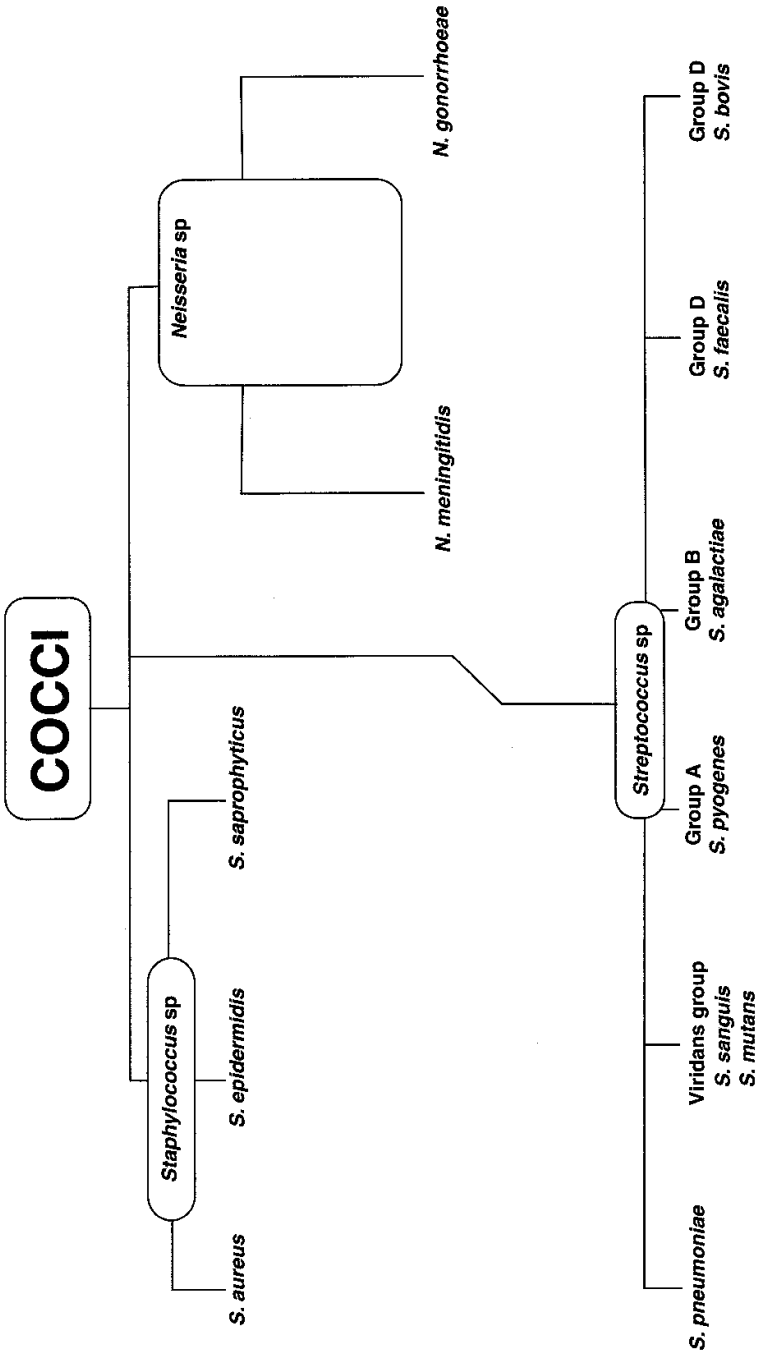
Mycoplasmas

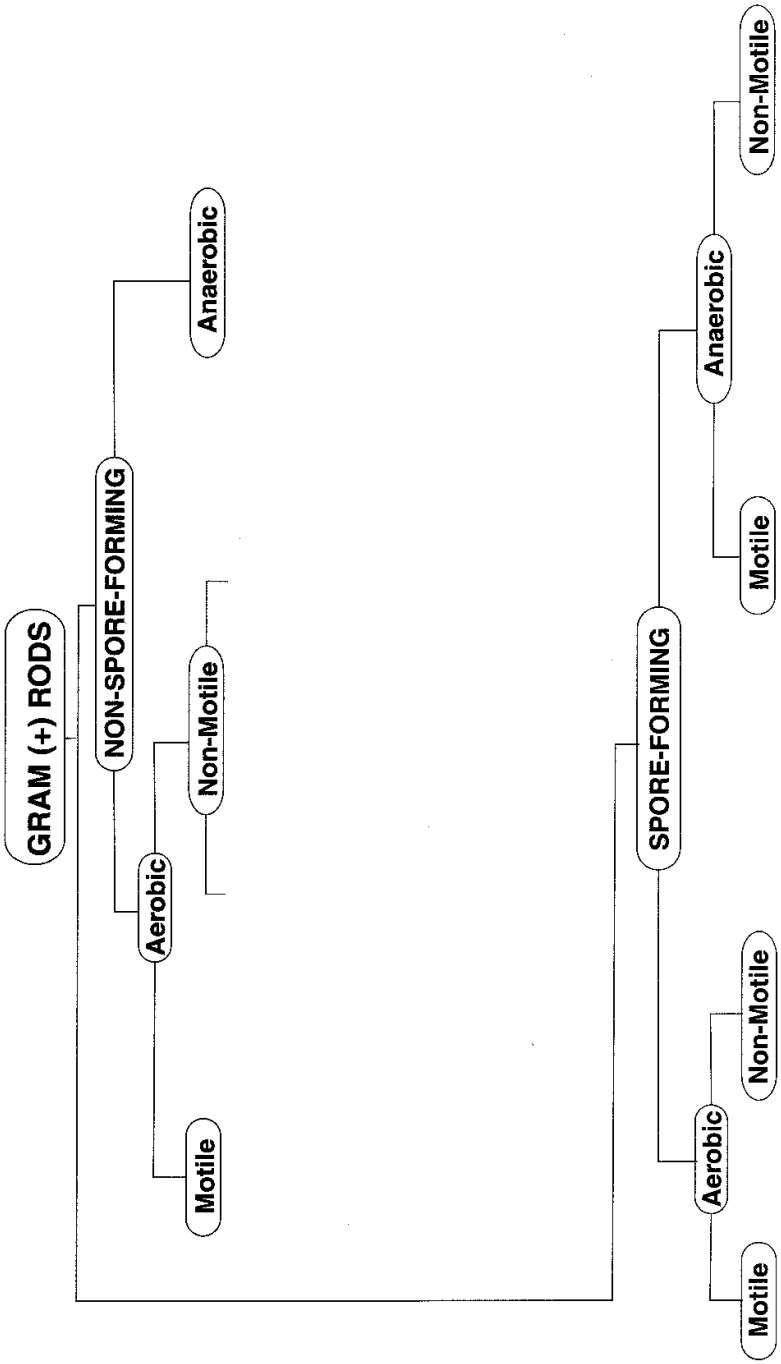
M. pneumoniae
- Lack cell wall peptidoglycan → non-Gram-staining
- Cholesterol (req'd) in membr.
- Atypical pneumonia in youth and young adults
- Free living (culturable, extracell.)
- Slow growth, special media: Mycoplasma, Eaton's or Hayflick's media-sterols+pur/pyrimidines: fried egg colonies
- Cold agglutinins in 65% cases
- No Penicillins nor Cephalosporins

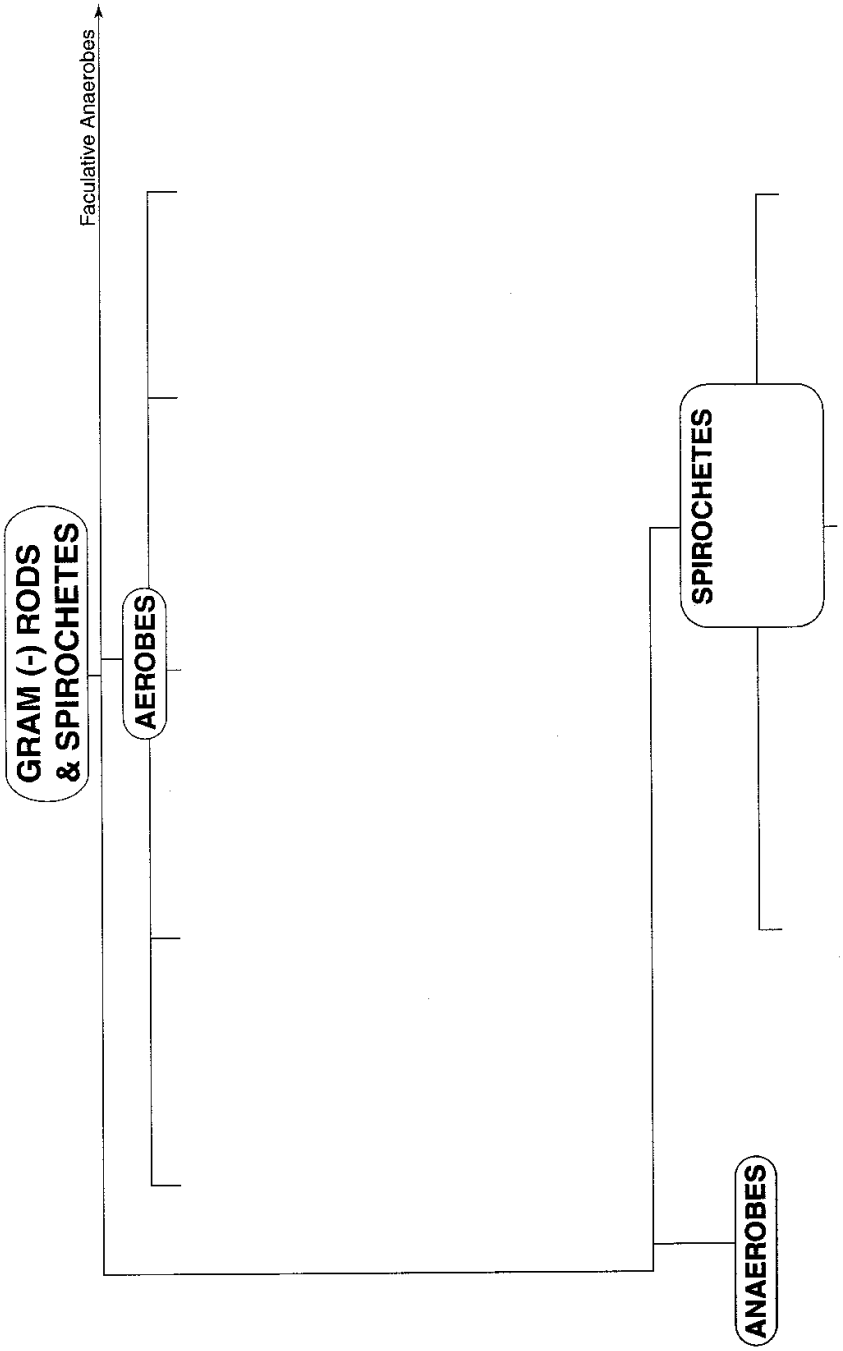
Ureaplasma urealyticum
- Urethritis, prostatitis
- Urease positive
- No cell wall

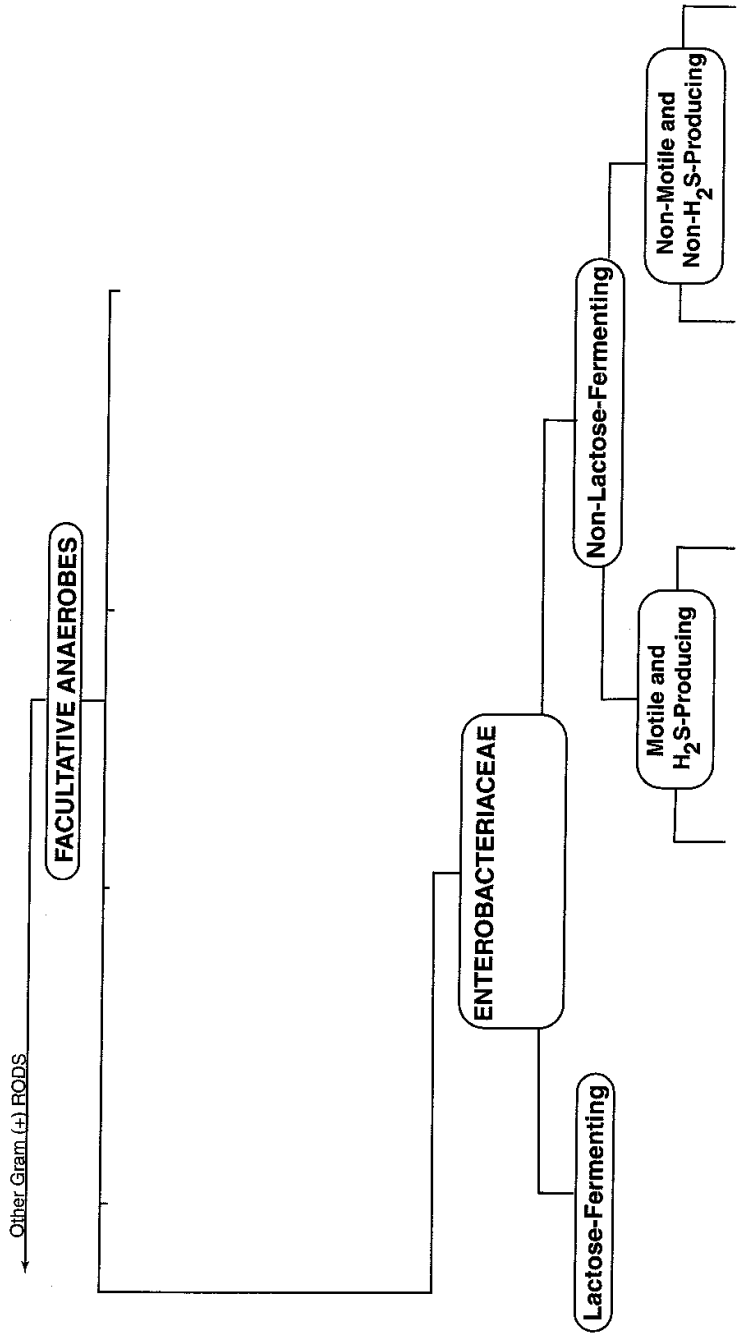
*Also note that *Legionella* and the spirochetes (*Treponema, Leptospira*, and *Borrelia*)—all Gram-negative—do not show up reliably with Gram stain.

COCCI
GRAM STRAIN
+
-
CATALASE
+
-
COAGULASE
+
-
NOVOBIOCIN
S
R
MALTOSE
fermentation
+
-
ALPHA-HEMOLYTIC
Partial (green) Hemolysis
BETA-HEMOLYTIC
Complete (clear) Hemolysis
GAMMA-HEMOLYTIC
Group D
Usually NO Hemolysis
OPTOCHIN
S
R
BACITRACIN
S
R
Growth in
6.5% NaCl

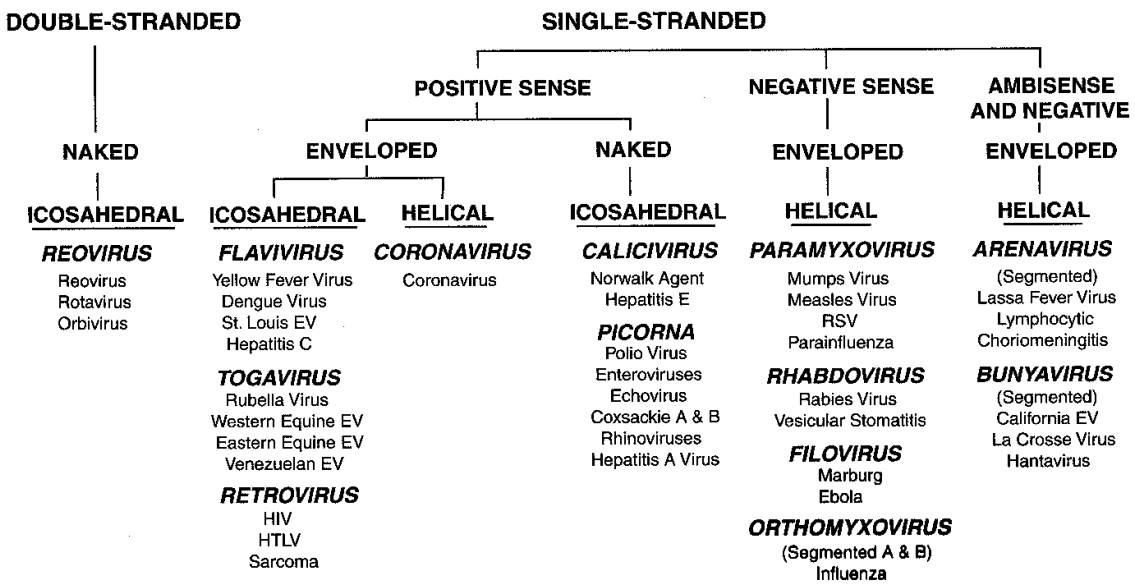

COCCI
Neisseria sp
N. gonorrhoeae
N. meningitidis
Staphylococcus sp
S. saprophyticus
S. epidermidis
S. aureus
Streptococcus sp
Group B
S. agalactiae
Group D
S. bovis
Group D
S. faecalis
Group A
S. pyogenes
Viridans group
S. sanguis
S. mutans
S. pneumoniae


GRAM (+) RODS
NON-SPORE-FORMING
Anaerobic
Aerobic
Non-Motile
Motile
SPORE-FORMING
Anaerobic
Non-Motile
Motile
Aerobic
Non-Motile
Motile

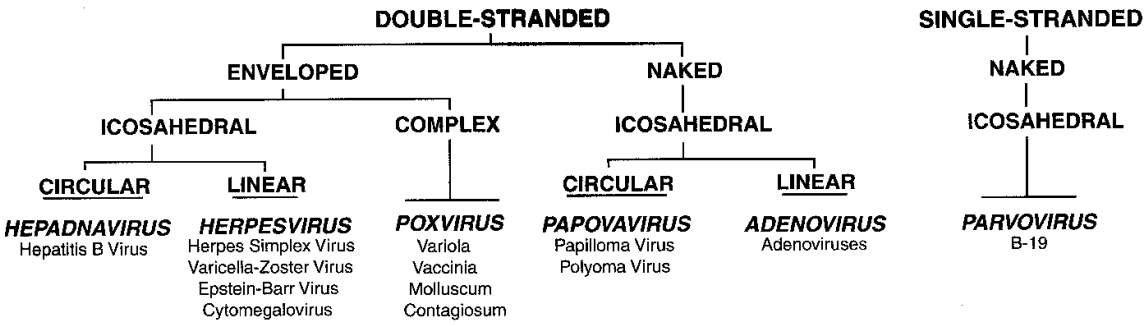

Faculative Anaerobes
GRAM (-) RODS & SPIROCHETES
AEROBES
SPIROCHETES
ANAEROBES

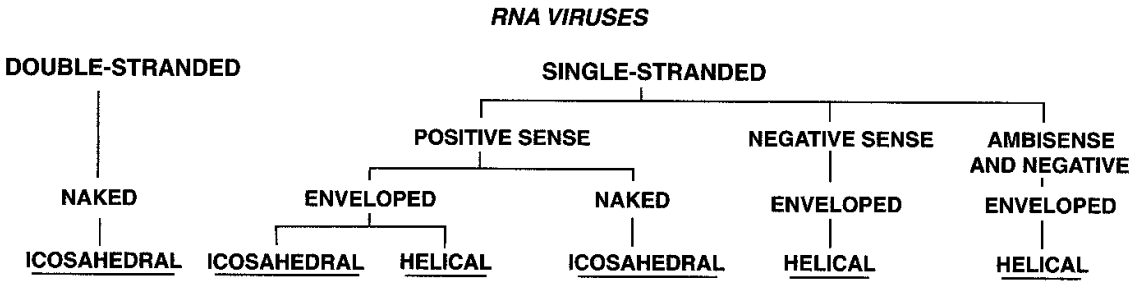

Other Gram (+) RODS
FACULTATIVE ANAEROBES
ENTEROBACTERIACEAE
Lactose-Fermenting
Non-Lactose-Fermenting
Motile and H_2S-Producing
Non-Motile and Non-H_2S-Producing

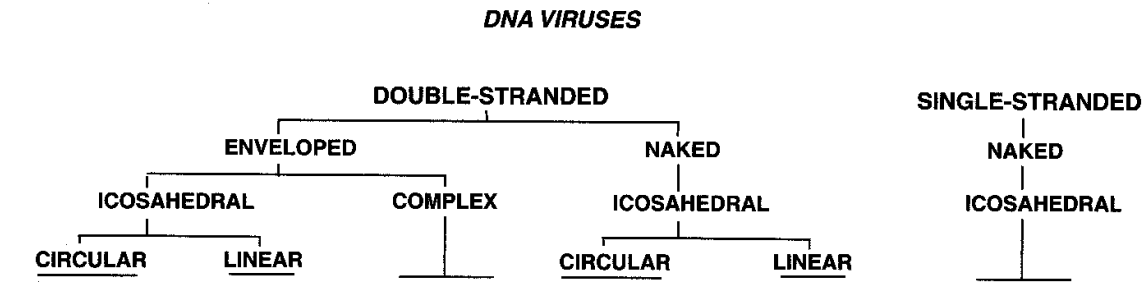
RNA VIRUSES



DNA VIRUSES




RNA VIRUSES
DOUBLE-STRANDED
NAKED
ICOSAHEDRAL
SINGLE-STRANDED
POSITIVE SENSE
ENVELOPED
ICOSAHEDRAL
HELICAL
NAKED
ICOSAHEDRAL
NEGATIVE SENSE
ENVELOPED
HELICAL
AMBISENSE AND NEGATIVE
ENVELOPED
HELICAL


DNA VIRUSES
DOUBLE-STRANDED
ENVELOPED
ICOSAHEDRAL
CIRCULAR
LINEAR
COMPLEX
NAKED
ICOSAHEDRAL
CIRCULAR
LINEAR
SINGLE-STRANDED
NAKED
ICOSAHEDRAL

SECTION II

Immunology

Overview of the Immune System

1

What the USMLE Requires You To Know

- Components of the innate and adaptive immune responses
- Attributes of innate and adaptive immune responses
- Interactions between innate and adaptive immune responses

The **immune system** is designed to produce a coordinated response to the introduction of foreign substances or **antigens** into the body. It is organizationally divided into two complementary arms: the **innate** (or **native** or **natural**) immune system and the **adaptive** (or **acquired** or **specific**) immune system.

Innate immunity provides the body's early line of defense against microbial invaders. It comprises four types of defensive barriers:

- **Anatomic** or physical (skin, mucous membranes)
- **Physiologic** (temperature, pH, and chemicals such as lysozyme, complement, and some interferons)
- **Phagocytic** (monocytes, neutrophils, macrophages)
- **Inflammatory** events

Innate immune defenses have in common that they:

- Are **present intrinsically** with or without previous stimulation
- Have **limited specificity** for shared structures of microbes
- Are **not enhanced** in activity by repeated exposure
- Have **limited diversity** of expression

Once the barriers of the innate immune response have been breached, the adaptive immune response is activated in an antigen-specific fashion to provide for the elimination of antigen and lasting protection from future challenge. The components of the adaptive immune system are:

- **Lymphocytes** (T cells and B cells) and plasma cells (end cells of B-lymphocyte differentiation)
- **Antigen-presenting cells** (macrophages, B cells, and dendritic cells)

In A Nutshell

The immune system has two arms:

- Innate
- Adaptive

In A Nutshell

The Innate Arm (Anatomic, Physiologic, Phagocytic, Inflammatory)

- Present intrinsically
- Nonspecific
- No memory
- Limited diversity

In A Nutshell

The Adaptive Arm (Lymphocytes and Their Products)

- Inducible
- Specific
- Memory
- Extensive diversity
- Self versus non-self distinction
- Self-limiting

Adaptive immune defenses have in common that they are:

- **Specific** for particular antigens and are specialized to provide the best protection
- **Diverse** in their specificity
- Enhanced with each repeated exposure (express **immunologic memory**)
- Capable of **self/non-self** recognition
- **Self-limiting**

These features of adaptive immunity are designed to give the individual the best possible defense against disease. **Specificity** is required, along with **memory**, to protect against persistent or recurrent challenge. **Diversity** is required to protect against the maximum number of potential pathogens. **Specialization** of function is necessary so that the most effective defense can be mounted against diverse challenges. The ability to distinguish between invaders and one's own cells and tissues (**self versus non-self**) is vital in inhibiting a response to one's own cells (**autoimmunity**). **Self-limitation** allows the system to return to a basal resting state after a challenge to conserve energy and prepare for the challenge by new microbes.

Table II-1-1. Comparison of Innate and Adaptive Immunity

Characteristics	Innate	Adaptive
Specificity	For structures shared by groups of microbes	For specific antigens of microbial and nonmicrobial agents
Diversity	Limited	High
Memory	No	Yes
Self-reactivity	No	No
Components		
Anatomic and chemical barriers	Skin, mucosa, chemicals (lysozyme, interferons α and β), temperature, pH	Lymph nodes, spleen, mucosal-associated lymphoid tissues
Blood proteins	Complement	Antibodies
Cells	Phagocytes and natural killer (NK) cells	Lymphocytes (other than NK cells)

In A Nutshell

- Antibodies and complement enhance phagocytosis.
- Antibodies activate complement.
- Cytokines stimulate adaptive and innate responses.

The innate and adaptive arms of the immune response do not operate independently of one another.

- Phagocytic cells process and display antigen to facilitate stimulation of specific T lymphocytes.
- Macrophages secrete immunoregulatory molecules (**cytokines**), which help trigger the initiation of specific immune responses.
- T lymphocytes produce cytokines, which enhance the microbicidal activities of phagocytes.
- Antibodies produced by plasma cells bind to pathogens and activate the complement system to result in the destruction of the invaders.
- Antibodies produced by B lymphocytes bind to pathogens and assist with phagocytosis (**opsonization**).

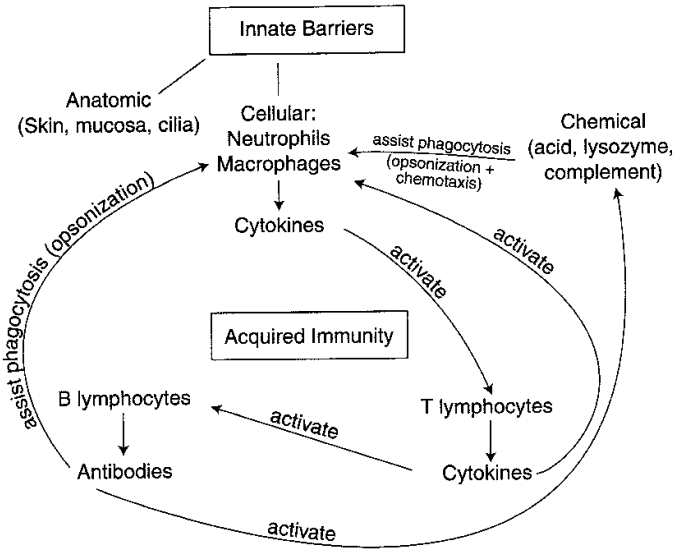


Figure II-1-1. Interaction Between Innate and Adaptive Immune Responses

Chapter Summary

- The immune system has two arms, innate and adaptive.
- The innate arm is a barrier system consisting of anatomic, physiologic, phagocytic, or inflammatory components.
- The innate arm is present intrinsically, has limited specificity and diversity, and is not enhanced by repeated exposure.
- The adaptive arm consists of T and B lymphocytes and antigen-presenting cells.
- Adaptive immune responses are specific, diverse, self-limiting, capable of self versus non-self recognition, and display memory.
- The innate and adaptive arms interact with and augment each other through soluble substances such as antibodies, complement, and cytokines.

Cells of the Immune System

What the USMLE Requires You To Know

- The cells of the immune system, their origin, tissue distribution, and function
- The structure and function of antigen-recognition molecules of B and T lymphocytes
- The make-up of the signal transduction complex of B and T lymphocytes
- The basic mechanism of gene-segment rearrangement to generate receptor diversity

ORIGIN

The cells of the immune system arise from a pluripotent stem cell in the bone marrow. Differentiation of this cell will occur along one of two pathways, giving rise to either a common lymphoid progenitor cell or a common myeloid progenitor cell. The common lymphoid progenitor cell gives rise to B lymphocytes, T lymphocytes, and natural killer (NK) cells. The myeloid progenitor gives rise to erythrocytes, platelets, basophils, mast cells, eosinophils, neutrophils, monocytes, macrophages, and dendritic cells.

In A Nutshell

- The lymphoid progenitor makes B cells, T cells, and NK cells.
- The myeloid progenitor makes red blood cells, platelets, basophils, mast cells, eosinophils, neutrophils, monocytes, macrophages, and dendritic cells.

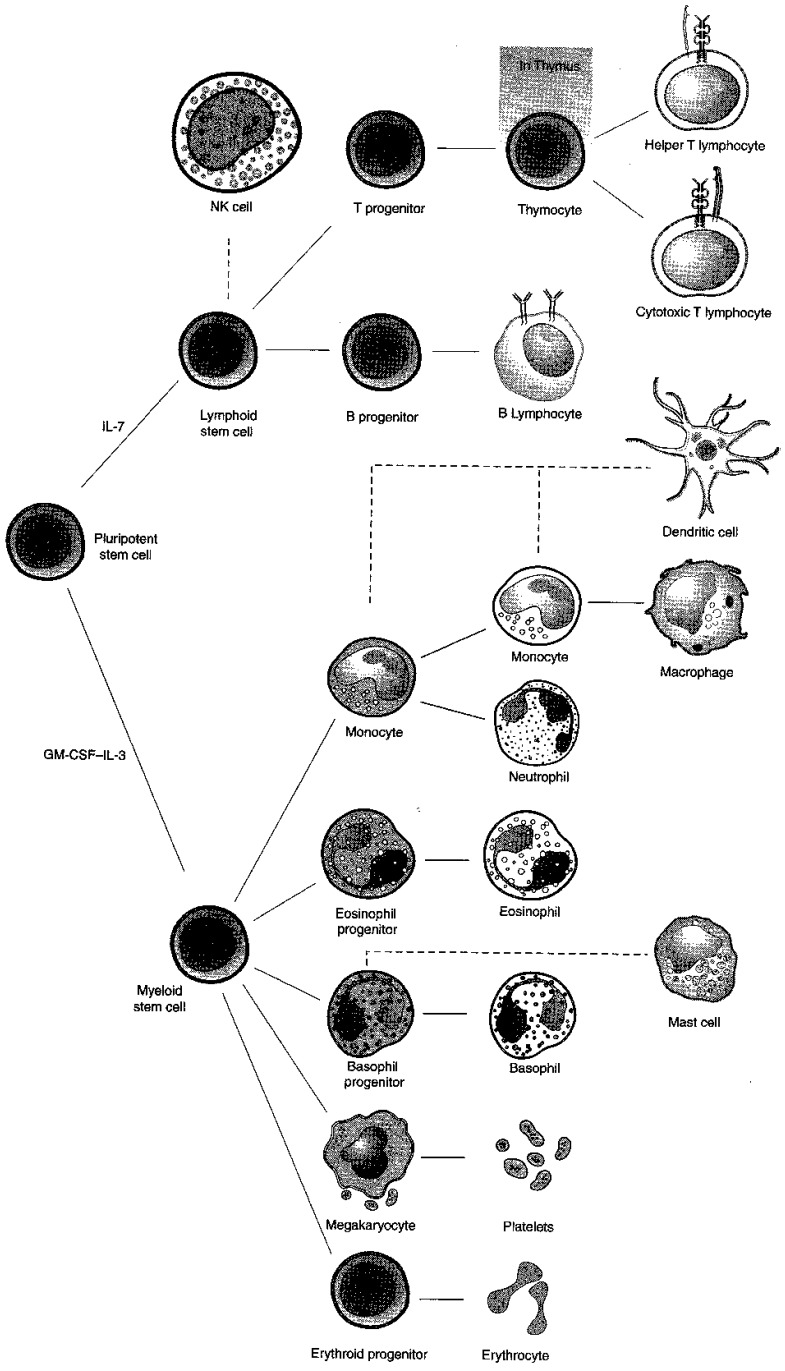
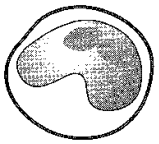
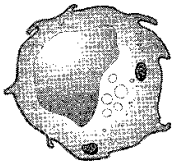
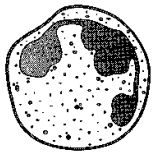
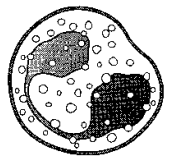


Figure II-2-1. The Ontogeny of Immune Cells

FUNCTION

The white blood cells of both myeloid and lymphoid stem cell origin have specialized functions in the body once their differentiation from the bone marrow is complete. Cells of myeloid heritage perform relatively stereotyped responses and are thus considered members of the innate branch of the immune system. Cells of the lymphoid lineage perform finely tuned, antigen-specific roles in immunity.

Table II-2-1. Myeloid Cells

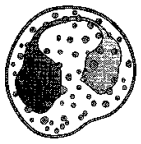
Myeloid Cell	Tissue Location	Identification	Function
Monocyte 	Bloodstream, 0–900/μL	Horseshoe-shaped nucleus	Phagocytic, differentiate into tissue macrophages
Macrophage 	Tissues	Ruffled membrane, cytoplasm with vacuoles and vesicles	Phagocytosis, secretion of cytokines
Dendritic cell	Epithelia, tissues	Long cytoplasmic arms	Antigen capture, transport, and presentation
Neutrophil 	Bloodstream, 1,800–7,800/μL	Multilobed nucleus; small pink granules	Phagocytosis and activation of bactericidal mechanisms
Eosinophil 	Bloodstream, 0–450/μL	Bilobed nucleus, large pink granules	Killing of antibody-coated parasites

(*Continued*)

In a Nutshell

- Myeloid cells are in the innate branch.
- Lymphoid cells (except NK cells) are in the adaptive branch.

Table II-2-1. Myeloid Cells (*continued*)

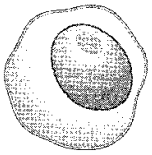
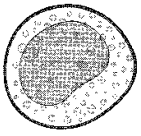
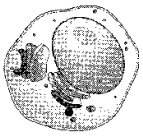
Myeloid Cell	Tissue Location	Identification	Function
Basophil 	Bloodstream, 0-200/μL	Bilobed nucleus, large blue granules	Nonphagocytic, release pharmacologically active substances during allergic responses
Mast cell	Tissues, mucosa, and epithelia	Small nucleus, cytoplasm packed with large blue granules	Release of granules containing histamine, etc., during allergic responses

In A Nutshell

- B lymphocytes are generated and mature in the bone marrow.
- T lymphocytes undergo maturation in the thymus.
- NK cells are large, granular lymphocytes.

Although lymphocytes in the bloodstream and tissues are nearly morphologically indistinguishable at the light microscopic level, we now know that there are several distinct but interdependent lineages of these cells: **B lymphocytes**, so called because they complete their development in the **bone marrow**, and **T lymphocytes**, so called because they pass from their origin in the bone marrow into the **thymus**, where they complete their development. Both have surface membrane–receptors designed to bind specific antigens. The third type of lymphocyte, the **natural killer (NK) cell**, is a **large, granular lymphocyte** that recognizes certain tumor and virus-infected cells (*See* Chapter 8).

Table II-2-2. Lymphoid Cells

Lymphoid Cell	Location	Identification	Function
Lymphocyte 	Bloodstream, 1,000–4,000/μl; lymph nodes, spleen, submucosa, and epithelia	Large, dark nucleus, small rim of cytoplasm	B cells produce antibody T helper cells regulate immune responses Cytotoxic T cells (CTLs) kill altered or infected cells
Natural killer (NK) lymphocyte 	Bloodstream, ≤10% of lymphocytes	Lymphocytes with large cytoplasmic granules	Kill tumor/virus cell targets or antibody-coated target cells
Plasma cell 	Lymph nodes, spleen, mucosal-associated lymphoid tissues, and bone marrow	Small dark nucleus, intensely staining Golgi apparatus	End cell of B-cell differentiation, produce antibody

THE ANTIGEN RECOGNITION MOLECULES OF LYMPHOCYTES

Each of the cells of the lymphoid lineage is now clinically identified by the characteristic surface molecules that they possess, and much is known about these structures, at least for B and T cells. The B lymphocyte, in its mature ready-to-respond form (the naive B lymphocyte), wears molecules of two types of antibody or immunoglobulin called IgM and IgD embedded in its membrane. The naive T cell wears a single type of genetically related molecule, called the T-cell receptor (TCR), on its surface. Both of these types of antigen receptors are encoded within the immunoglobulin superfamily of genes and are expressed in literally millions of variations in different lymphocytes as a result of complex and random rearrangements of the cells' DNA.

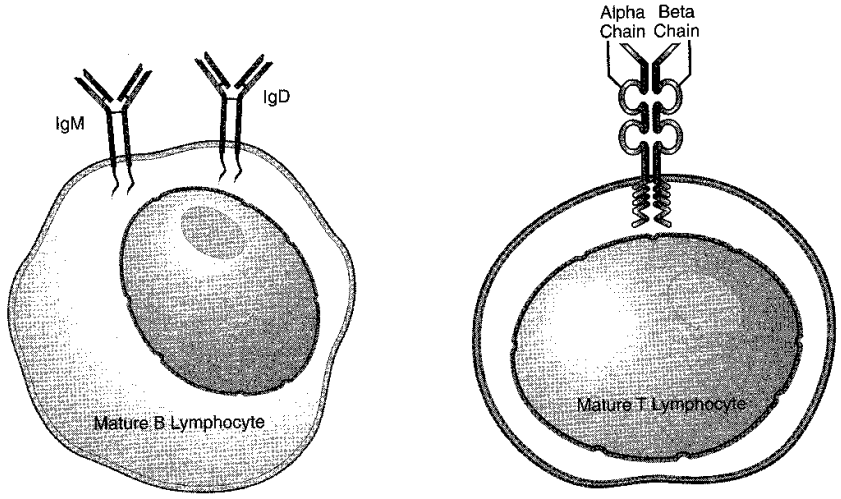


Figure II-2-2. Antigen Receptors of Mature Lymphocytes

The antigen receptor of the B lymphocyte, or **membrane-bound immunoglobulin**, is a four-chain glycoprotein molecule that serves as the basic monomeric unit for each of the distinct antibody molecules destined to circulate freely in the serum. This monomer has two identical halves, each composed of a long, or **heavy chain** (μ for immunoglobulin [Ig] M and δ for IgD), and a shorter, **light chain** (κ or λ). A cytoplasmic tail on the carboxy-terminus of each heavy chain extends through the plasma membrane and anchors the molecule to the cell surface. The two halves are held together by disulfide bonds into a shape resembling a "Y," and some flexibility of movement is permitted between the halves by disulfide bonds forming a **hinge region**. On the N-terminal end of the molecule where the heavy and light chains lie side by side, a "pocket" is formed whose three-dimensional shape will accommodate the noncovalent binding of one, or a very small number, of related antigens. The unique three-dimensional shape of this pocket is called the **idiotype** of the molecule, and although two classes (**isotypes**) of membrane immunoglobulin (IgM and IgD) are coexpressed (defined by amino acid sequences toward the carboxy terminus of the molecule), only one idiotype or **antigenic specificity** is expressed per cell (although in multiple copies). Each human individual is capable of producing hundreds of millions of unique idiotypes.

In a Nutshell

- The naive B-cell antigen receptors are IgM and IgD.
- The T-cell antigen receptor is made of α and β chains.

In A Nutshell

- Membrane-bound Ig has two heavy and two light chains.
- A "hinge" region joins the heavy chains.
- The idiotype of the molecule resides in the N-terminal pocket of heavy and light chains.
- The isotype of the molecule is determined by domains toward the C-terminus.

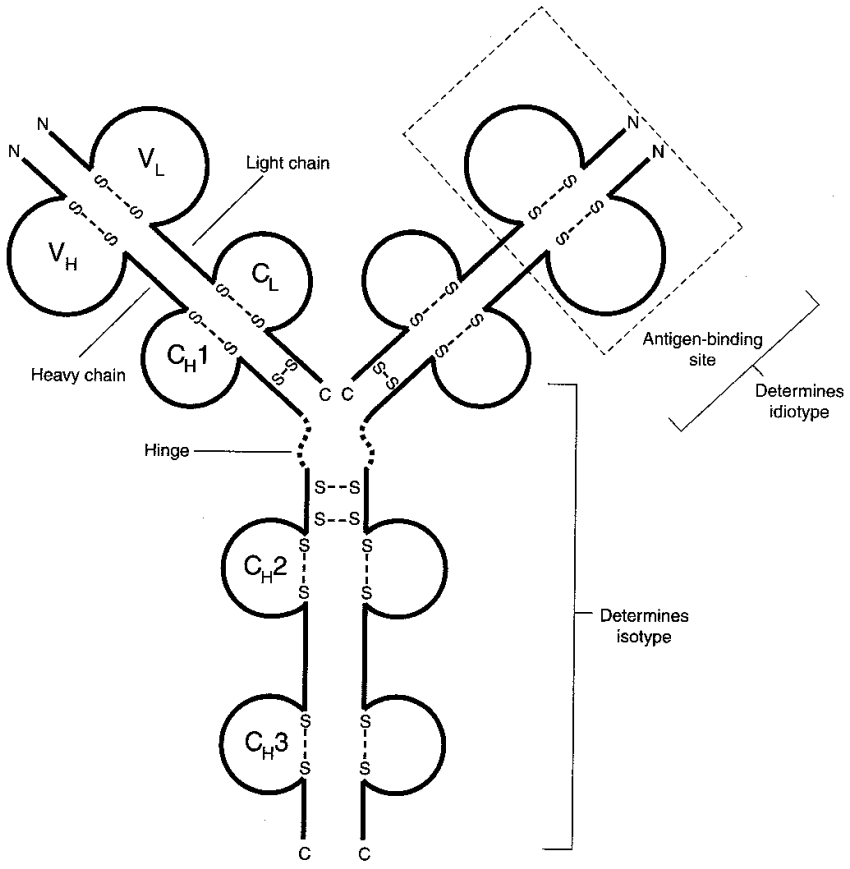


Figure II-2-3. The B-Lymphocyte Antigen Recognition Molecule (Membrane-Bound Immunoglobulin)

The antigen receptor of the T lymphocyte is composed of two glycoprotein chains that are similar in length and are thus designated α and β chains. A small proportion of T cells expresses an alternative TCR composed of γ and δ chains, but the function of these cells is poorly understood. On the carboxy-terminus of α/β chains, a cytoplasmic tail extends through the membrane for anchorage. On the N-terminal end of the molecule, a groove is formed between the two chains, whose three-dimensional shape will accommodate the binding of a small antigenic **peptide** presented on the surface of an antigen-presenting cell (macrophage, dendritic cell, or B lymphocyte). This groove forms the idiotype of the TCR. Notice that there is no hinge region present in this molecule, and thus its conformation is quite rigid.

The membrane receptors of B lymphocytes are designed to bind **unprocessed antigens** of almost any chemical composition, whereas the TCR is designed to bind only **cell-bound peptides.** Also, although the B-cell receptor is ultimately modified to circulate freely in the plasma as secreted **antibody**, the TCR is never released from its membrane-bound location.

In association with these unique antigen-recognition molecules on the surface of B and T cells, accessory molecules are found whose function is in signal transduction. Thus, when a lymphocyte

In A Nutshell

- The T-cell receptor has α/β chains.
- It binds peptides presented by antigen-presenting cells.
- The molecule is rigid.
- The molecule is always cell-bound.

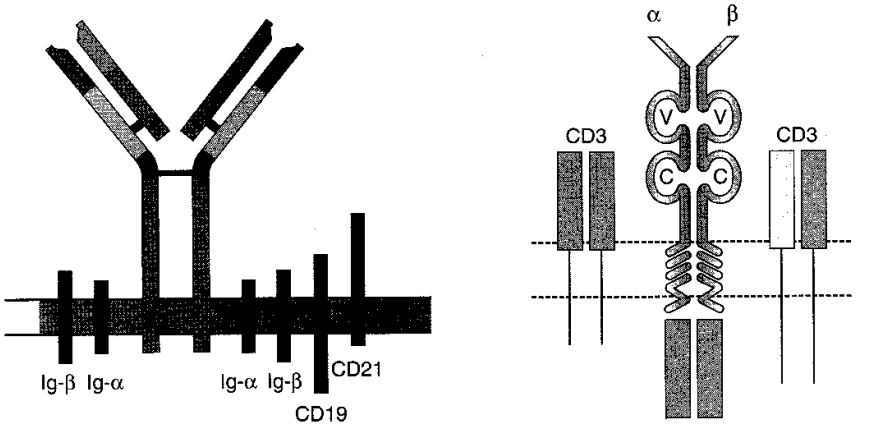
In A Nutshell

- B cells recognize unprocessed antigens.
- T cells recognize cell-bound peptides.

In A Nutshell

- The B-cell signal transduction complex is Ig-α, Ig-β, CD19, and CD21.
- The T-cell signal transduction complex is CD3.

binds to an antigen complementary to its idiotype, a cascade of messages transferred through its **signal transduction complex** will culminate in intracytoplasmic phosphorylation events, which will activate the cell. In the B cell, this signal transduction complex is composed of two single-chain immunoglobulin relatives known as Ig-α and Ig-β and two other molecules designated CD (cluster of differentiation) 19 and 21. In the T cell, the signal transduction complex is a multichain structure called CD3.



B-Cell Signal Transduction Complex **T-Cell Signal Transduction Complex**

Figure II-2-4

Table II-2-3. Comparison of B- and T-Lymphocyte Antigen Receptors

Property	B-Cell Antigen Receptor	T-Cell Antigen Receptor
Molecules/Lymphocyte	100,000	100,000
Idiotypes/Lymphocyte	1	1
Isotypes/Lymphocyte	2 (IgM and IgD)	1 (α/β)
Is secretion possible?	Yes	No
Number of combining sites/molecule	2	1
Mobility	Flexible (hinge region)	Rigid
Signal-transduction molecules	Ig-α, Ig-β, CD19, CD21	CD3

THE GENERATION OF RECEPTOR DIVERSITY

Because the body requires the ability to respond specifically to all of the millions of potential harmful agents it may encounter in a lifetime, a mechanism must exist to generate the millions of idiotypes of antigen receptors necessary to meet this challenge. If each of these idiotypes were encoded separately in the germline DNA of lymphoid cells, it would require more DNA than is present in the entire cell. The generation of this necessary diversity is accomplished by a complex and unique set of rearrangements of DNA segments that takes place during the maturation of lymphoid cells.

In A Nutshell

- Millions of distinct idiotypes are generated by rearranging gene segments, which code for the variable domains of the B- or T-cell receptors.
- Three gene segments (V, D, and J) are combined to create the variable domain of the B cell heavy chain or the TCR β chain.

In the first place, it was discovered that individuals inherit a large number of different segments of DNA, which may be recombined and alternatively spliced to create unique amino acid sequences in the N-terminal ends (**variable domains**) of the chains that compose their antigen recognition sites. For example, to produce the **heavy chain variable domains** of their antigen receptor, B-lymphocyte progenitors select randomly and in the absence of stimulating antigen to recombine three gene segments designated variable (V), diversity (D), and joining (J) out of hundreds of germline-encoded possibilities to produce unique sequences of amino acids in the variable domains (VDJ recombination). An analogous random selection is made during the formation of the β chain of the TCR.

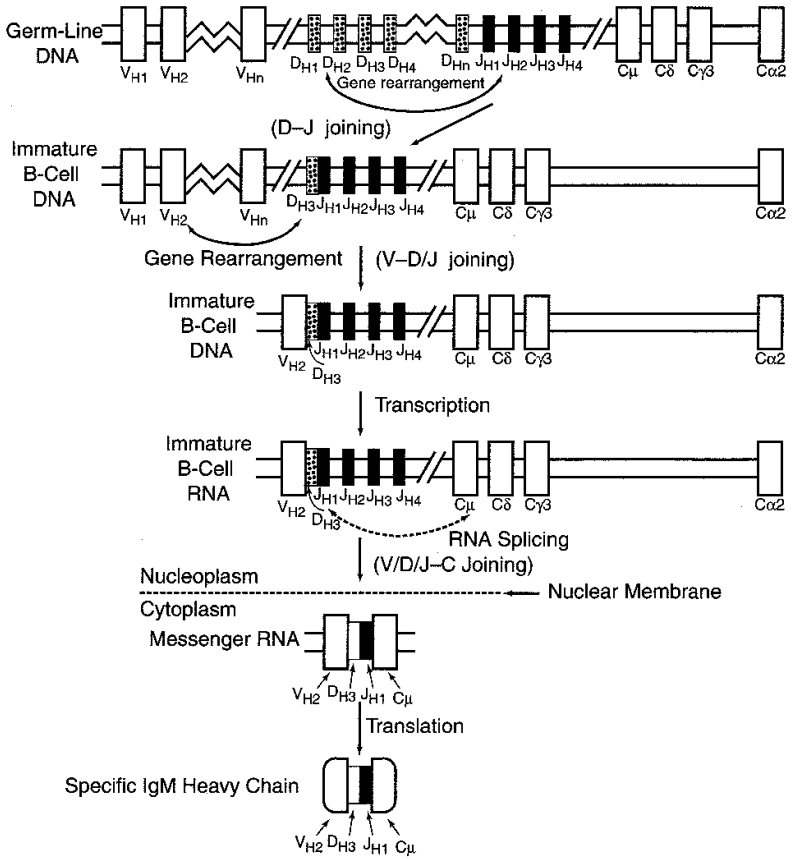


Figure II-2-5. Production of Heavy (B-Cell) or Beta (T-Cell) Chains of Lymphocyte Antigen Receptors

In A Nutshell

Two gene segments (V and J) are combined to create the B-cell light chain or the TCR-α chain of the T-cell receptor.

Next, the B-lymphocyte progenitor performs random rearrangements of two types of gene segments (V and J) to encode the **variable domain amino acids of the light chain.** An analogous random selection is made during the formation of the α chain of the TCR.

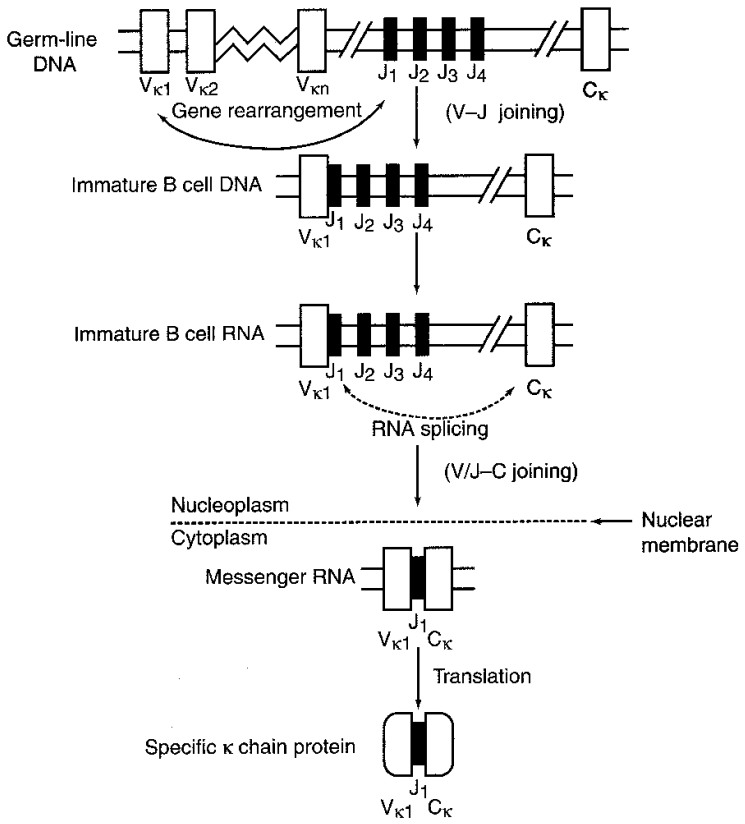


Figure II-2-6. Production of Light (B Cell) or Alpha (T Cell) Chain of a Lymphocyte Antigen Receptor

While heavy chain gene segments are undergoing recombination, the enzyme **terminal deoxyribonucleotidyl transferase** (Tdt) randomly inserts bases (without a template on the complementary strand) at the junctions of V, D, and J segments (N-nucleotide addition). When the light chains are rearranged later, Tdt is not active, but it is active during the rearrangement of all gene segments in the formation of the TCR. This generates even more diversity than the random combination of V, D, and J segments alone.

Needless to say, many of these gene segment rearrangements result in the production of truncated or nonfunctional proteins. When this occurs, the cell has a second chance to produce a functional strand by rearranging the gene segments of the homologous chromosome. If it fails to make a functional protein from rearrangement of segments on either chromosome, the cell is induced to undergo **apoptosis** or programmed cell death. In this way, the cell has two chances to produce a functional heavy (or β) chain. A similar process occurs with the light or α chain. Once a functional product has been achieved by one of these rearrangements, the cell shuts off the rearrangement and expression of the other allele on the homologous chromosome—a process known as **allelic exclusion**. This process ensures that B and T lymphocytes synthesize only **one specific antigen-receptor per cell.**

In A Nutshell

- The enzyme Tdt inserts bases randomly at the junctions of V, D, and J and creates more variability.
- Once a functional product has been made, the homologous chromosome is inactivated (allelic exclusion).

Clinical Correlate

Tdt is used as a marker for early stage T- and B-cell development in acute lymphoblastic leukemia. (*See* Chapter 15.)

Because any heavy (or β) chain can associate with any randomly generated light (or α) chain, one can multiply the number of different possible heavy chains by the number of different possible light chains to yield the total number of possible idiotypes that can be formed. This generates yet another level of diversity.

In A Nutshell

- The products of variable domain rearrangements are alternatively spliced at the RNA level to IgM or IgD constant domains to form the B-cell antigen receptor.
- IgM and IgD molecules with identical idiotypes are expressed on mature B cells.

Table II-2-4. Summary of Mechanisms for Generating Receptor Diversity

Mechanism	Cell in Which It Is Expressed
Existence in genome of multiple V, D, J segments	B and T cells
VDJ recombination	B and T cells
N-nucleotide addition	B cells (only heavy chain) T cells (all chains)
Combinatorial association of heavy and light chains	B and T cells
Somatic hypermutation	B cells only, after antigen stimulation (*see* Chapter 7)

Downstream on the germline DNA from the coding segments, which have now been rearranged to yield the variable domain that will serve as the antigen-combining site of the molecule, are encoded in sequence, the amino acid sequences of all of the remaining domains of the chain. These domains tend to be similar within the classes or isotypes of immunoglobulin or TCR chains and are thus called constant domains. The first set of constant domains for the heavy chain of immunoglobulin that is transcribed is that of IgM and next, IgD. These two sets of domains are alternatively spliced to the variable domain product at the RNA level. There are only two isotypes of light chain constant domains, named κ and λ, and one will be combined with the product of light chain variable domain rearrangement to produce the other half of the final molecule. Thus, the B lymphocyte produces IgM and IgD molecules with identical idiotypes and inserts these into the membrane for antigen recognition.

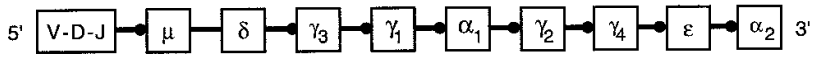


Figure II-2-7. Immunoglobulin Heavy Chain DNA

Chapter Summary

- The cells of the immune system arise from a pluripotent stem cell in the **bone marrow**.
- The common lymphoid progenitor will give rise to B lymphocytes, T **lymphocytes, and NK cells**.
- The common myeloid progenitor will give rise to erythrocytes, platelets, **basophils, mast cells,** eosinophils, neutrophils, monocytes, macrophages, and dendritic cells.
- The phagocytic cells of the myeloid series include monocytes, macrophages, **dendritic cells,** neutrophils, and eosinophils.
- Basophils and mast cells are nonphagocytic cells, which mediate allergic responses.
- B lymphocytes secrete immunoglobulin; T cells may be helper or killer cells; and NK cells kill tumor or virus-infected target cells.
- Plasma cells are the end cells of B-lymphocyte differentiation and secrete antibody.
- The antigen receptor of the B lymphocyte is membrane-bound IgM and IgD and is designed to bind unprocessed antigens of almost any chemical composition.

The antigen receptor of the T lymphocyte is composed of two chains (α/β or γ/δ) and is designed to recognize cell-bound peptides.

- B-cell antigen receptors can be secreted, whereas T-cell receptors are always cell-bound.
- The antigen receptors of B and T cells are associated with signal transduction molecules: Igα, Igβ, CD19, and CD21 for B cells and CD3 for T cells.
- The diversity of idiotypes of antigen-combining sites is generated by rearrangements of gene segments coding for variable domain amino acids and is assisted by the action of the enzyme terminal deoxyribonucleotidyl transferase.
- There are two major points when considering gene rearrangement:

  1) The difference between the heavy and light chains is the presence of the D region in the heavy chain, and

  2) Only rearranged genes can actually be expressed; therefore, only lymphocytes express antigen receptors.

- Allelic exclusion is the process by which one chromosome of a homologous pair will be inactivated, and it ensures that only one idiotype of antigen-recognition molecule will be produced per cell.

Review Questions

1. A germline B lymphocyte possesses 200 distinct V region genes, 5 J region genes, and 2 isotypic possibilities to rearrange for its selection of light chain synthesis. Assuming no recombinational inaccuracies, how many distinct idiotypes could be produced by combining this coding sequence with one heavy chain?

   (A) 10
   (B) 205
   (C) 400
   (D) 1000
   (E) 2000

2. Isotype switching during B-cell ontogeny dedicates mature B cells to production of a single heavy chain isotype, except in the case of IgM and IgD, which can be expressed concomitantly. How is this expression of both isotypes simultaneously possible?

   (A) Allelic exclusion
   (B) Allelic codominance
   (C) Affinity maturation
   (D) Alternative RNA splicing
   (E) Somatic hypermutation

3. A 4-year-old Caucasian boy is brought to his pediatrician with complaints of abnormal bruising and repeated bacterial infections. A blood workup reveals thrombocytopenia and neutropenia and the presence of numerous small, dense lymphoblasts with scant cytoplasm. Immunophenotyping of the abnormal cells determines them to be extremely primitive B cells, which are CD19+, HLA-DR+, and Tdt+. Which of the following best describes the status of immunoglobulin chain synthesis most likely in these cells?

   (A) IgM heavy chains inserted in the membrane
   (B) IgM heavy chains present in the cytoplasm
   (C) IgM monomers inserted in the membrane
   (D) IgM monomers present in the cytoplasm
   (E) No immunoglobulin chain synthesis present

4. A young woman with acute myeloblastic leukemia is treated with intensive chemotherapy and achieves remission of her symptoms. Because the prognosis for relapse is relatively high, a bone marrow transplant is undertaken in her first remission. Which of the following cytokines administered with the bone marrow cells would have the beneficial result of stimulating lymphoid-cell development from the grafted stem cells?

   (A) Interleukin (IL)-1
   (B) IL-2
   (C) IL-3
   (D) IL-6
   (E) IL-7

5. A 2-year-old boy is evaluated for a severe combined immunodeficiency disease. His bone marrow has normal cellularity. Radioactive tracer studies demonstrate a normal number of T-cell precursors entering the thymus, but no mature T lymphocytes are found in the blood or peripheral organs. Cells populating the thymus are found to lack CD3. Which of the following capabilities would his cells lack?

   (A) Ability to bind cell-bound peptides
   (B) Ability to express CD4/CD8 coreceptors
   (C) Ability to produce terminal deoxyribonucleotidyl transferase
   (D) Ability to proliferate in response to specific antigen
   (E) Ability to rearrange T-cell receptor gene segments

Answers and Explanations

1. **The correct answer is D.** The portion of the light chain that will be found within the antigen-combining site (idiotype) of an antibody molecule is formed by random rearrangement of V and J gene segments. Thus, given the numbers here, there are 200 × 5 different possible combinations. The isotypic (constant domain) possibilities do not play a part in the formation of the idiotype.

   Choice A, 10, is not correct. If you selected this answer, you multiplied the number of J region genes times the number of isotypes. This is not a recombination that would produce the idiotype.

   Choice B, 205, is not correct. If you selected this answer, you added the number of V region and J region genes together. Although you chose the correct gene segments to recombine, remember that the number of possible combinations of 200 choices and 5 choices requires that you *multiply*, not add, those figures.

   Choice C, 400, is not correct. If you selected this answer, you multiplied the number of V region genes times the number of isotypic possibilities. This is not a recombination that would produce the idiotype.

   Choice E, 2,000, is not correct. If you selected this answer, you multiplied the number of V region genes times the number of J region genes (to this point you were correct), but then further multiplied by the number of isotypic possibilities. The isotypic possibilities do not play a part in the formation of the idiotype.

2. **The correct answer is D.** Alternative RNA splicing allows a mature B cell to attach either δ or μ chains on a single idiotype that has been generated by germ-line DNA rearrangements.

   Allelic exclusion **(choice A)** refers to the expression of products of either parental chromosome type, but not both. This allows lymphoid cells to express only one type of antigen receptor (one idiotype) per cell and is essential to cellular specificity of action.

   Allelic codominance **(choice B)** refers to the expression of products of both parental chromosomes simultaneously. It is found in the expression of MHC class I and II products, but not in the expression of immunoglobulin gene products.

   Affinity maturation **(choice C)** refers to the increase of affinity (binding strength) of a population of antibodies over time during the development of an immune response. Because the affinity of an antibody is dependent on the goodness-of-fit of its idiotype for its antigen, isotype switching does not affect the shape of the idiotype and does not change the affinity of the molecule.

   Somatic hypermutation **(choice E)** is the phenomenon that allows affinity maturation to occur. It is the accelerated mutation of DNA coding within the hypervariable region that occurs during B-cell proliferation in response to antigenic stimulation. Again, the isotype of the antibody does not affect the shape of the idiotype, and this term refers to a process that changes the shape of the idiotype.

3. **The correct answer is E.** This child has acute lymphoblastic leukemia (ALL), and the malignant cells have the characteristics of early B-cell precursors. This leukemia has peak incidence at approximately 4 years of age, is twice as common in whites than in nonwhites, and is slightly more frequent in boys than in girls. A leukemic cell that is positive for terminal deoxyribonucleotidyl transferase (Tdt) is in the process of rearranging the gene segments for synthesis of the heavy chain of immunoglobulin but will not yet have completed a functional product. Tdt is active for all heavy-domain gene segment rearrangements but is not used during light-chain gene segment rearrangements.

IgM heavy chains inserted in the membrane (**choice A**) would be found in cells that have completed the rearrangement of their heavy chain variable domain gene segments, and these may transiently be expressed on the surface of a cell in association with a surrogate light chain before light chain rearrangement is complete. These cells would not be using their Tdt any more.

IgM heavy chains in the cytoplasm (**choice B**) would be found in leukemic cells that are more highly differentiated than those described. Once the variable domain gene segments for the heavy chain have been successfully rearranged in a cell, μ chains can be found in the cytoplasm. In ALL, this is usually associated with a decreased expression of Tdt and appearance of CD10 (the common acute lymphoblastic leukemia antigen; CALLA) and CD20.

IgM monomers inserted in the membrane (**choice C**) would be found in leukemic cells that are at the mature B-cell stage. Such cells would have completed the rearrangements for both heavy and light chains and would lack Tdt as a marker. They would express surface HLA-DR, CD19, and CD20 in addition to surface immunoglobulin.

IgM monomers present in the cytoplasm (**choice D**) would be found in cells that have completed the rearrangement of their variable domain gene segments. They would no longer express Tdt.

4. **The correct answer is E.** The cytokine most strongly associated with stimulation of production of lymphoid cells from the bone marrow is interleukin (IL)-7.

   IL-1 (**choice A**) is the endogenous pyrogen. It is produced by macrophages and acts on the hypothalamus to raise the temperature set point. It is associated with systemic inflammatory processes, but is not known to have an effect on lymphopoiesis.

   IL-2 (**choice B**) is a product of T cells that stimulates proliferation of T cells in the periphery. It is not known to have an effect on lymphopoiesis.

   IL-3 (**choice C**) is the cytokine that is most strongly associated with stimulation of myeloid cell precursors in the bone marrow.

   IL-6 (**choice D**) is a second endogenous pyrogen. It causes production of acute-phase proteins from hepatocytes and acts on myeloid stem cells in the bone marrow to induce differentiation.

5. **The correct answer is D.** CD3 is the signal transduction complex in T lymphocytes. When specific antigen binding has occurred on the surface of the cell, this complex is responsible for transferring the message to the cytoplasm of the cell. This culminates in intracytoplasmic phosphorylation events, which activate the cell and induce its proliferation (cloning). A cell lacking CD3 would be capable of binding specific antigen, but incapable of activation and proliferation in response to that first signal.

   Ability to bind cell-bound peptides (**choice A**) would not be affected by the absence of CD3. Binding to peptides presented by antigen-presenting cells is through interaction of the T-cell receptor with major histocompatibility antigens on the surface of other cells.

   Ability to express coreceptors (**choice B**) would not be affected by the absence of CD3, although cells would not be able to complete their differentiation in the thymus and become fully committed T cells.

   Ability to produce terminal deoxyribonucleotidyl transferase (**choice C**) would not be affected by the absence of the T-cell signal transduction complex. T-cell precursors rearrange their receptor gene segments (and use terminal deoxyribonucleotidyl transferase) in the absence of antigenic stimulation and before signal transduction through CD3 becomes critical.

Ability to rearrange T-cell receptor gene segments (**choice E**) would not be affected by the absence of the T-cell signal transduction complex. T-cell precursors rearrange their receptor gene segments in the absence of antigenic stimulation and before signal transduction through CD3 becomes critical.

The Selection of Lymphocytes

3

What The USMLE Requires You To Know

- The primary lymphoid organs: structure and function
- The ontogeny of T- and B-lymphocyte cell surface markers
- The structure and function of MHC gene products

As lymphoid progenitors develop in the bone marrow, we have seen that they make random rearrangements of their germline DNA to produce the unique idiotypes of antigen-recognition molecules that they will use throughout their lives. The bone marrow, therefore, is considered a **primary lymphoid organ** in humans because it supports and encourages these early developmental changes. B lymphocytes complete their entire formative period in the bone marrow and can be identified in their progress by the immunoglobulin chains they produce.

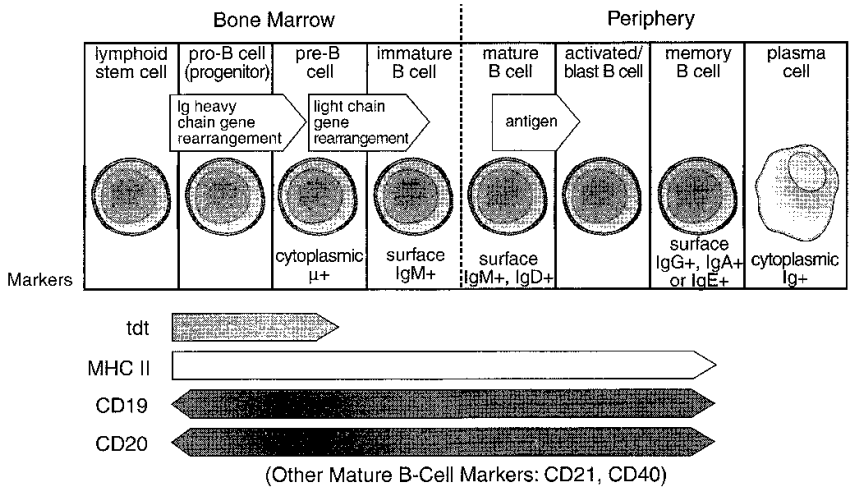


Figure II-3-1. B-Cell Differentiation

Because these gene segment rearrangements occur randomly and in the absence of stimulation with foreign antigen, it stands to reason that many of the idiotypes of receptors produced could have a binding attraction or **affinity** for normal body constituents These cells, if allowed to develop further, could develop into self-reactive lymphocytes that could cause harm to the host.

In A Nutshell

Primary lymphoid organs are sites of lymphoid-cell development (lymphopoiesis).

- Bone marrow
- Thymus

Secondary lymphoid organs are sites of antigen exposure.

- Spleen
- Lymph nodes
- Mucosal-associated lymphoid tissues

In A Nutshell

Clonal anergy and clonal deletion produce self-tolerance.

Therefore, one of the key roles of the bone marrow stroma and interdigitating cells is to remove such potentially harmful products. Cells whose idiotype has too great an affinity for normal cellular molecules are either deleted in the bone marrow (**clonal deletion**) or inactivated in the periphery (**clonal anergy**). In such a way, only those cells that are **selectively unresponsive** (**tolerant**) to self-antigens are allowed to leave the bone marrow.

In A Nutshell

T-cell precursors leave the bone marrow to undergo selection and maturation in the thymus.

Immature lymphocytes destined to the T-cell lineage leave the bone marrow and proceed to the **thymus**, the second **primary lymphoid organ** dedicated to the maturation of T cells. The thymus is a bilobed structure located above the heart that consists of an outer **cortex** packed with immature T cells and an inner **medulla** into which cells pass as they mature. Both the cortex and medulla are laced with a network of epithelial cells, dendritic cells, and macrophages, which interact physically with the developing thymocytes.

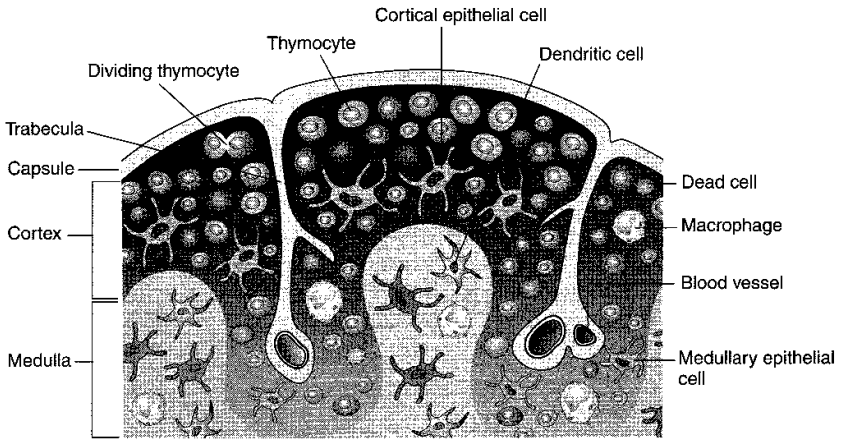


Figure II-3-2. The Structure of the Thymus

In A Nutshell

Thymocytes are exposed to MHC class I and II antigens.

As the developing thymocytes begin to express their TCRs, they are subjected to a rigorous two-step selection process. Because the TCR is designed to bind antigenic peptides presented on the surface of **antigen-presenting cells** (APCs) in the body, a selection process is necessary to remove those cells that would bind to normal self antigens and cause **autoimmunity,** as well as those that have no attraction whatsoever for the surfaces of APCs. This is accomplished by exposure of developing thymocytes to high levels of a unique group of membrane-bound molecules known as **major histocompatibility complex** (MHC) antigens.

The MHC is a collection of highly polymorphic genes on the short arm of chromosome 6 in the human. There are two classes of cell-bound MHC gene products (classes I and II). Both class I and class II molecules are expressed at high density on the surface of cells of the thymic stroma.

In A Nutshell

MHC Class I

- α chain plus β_2-microglobulin
- Codominantly expressed
- All nucleated cells of the body

Table II-3-1. Class I and II Gene Products

Class I Gene Products			Class II Gene Products		
HLA-A	HLA-B	HLA-C	HLA-DP	HLA-DQ	HLA-DR

Class I molecules are expressed on all nucleated cells in the body, as well as platelets. They are expressed in codominant fashion, meaning that each cell expresses two A, two B, and two C products (one from each parent). The molecules (A, B, and C) consist of an α heavy chain with

three extracellular domains and an intracytoplasmic carboxy-terminus. A second light chain, β_2-microglobulin, is not encoded within the MHC and functions in transport of the class I antigen to the cell surface. A groove between the first two extracellular domains of the α chain is designed to accommodate small peptides to be presented to the TCR.

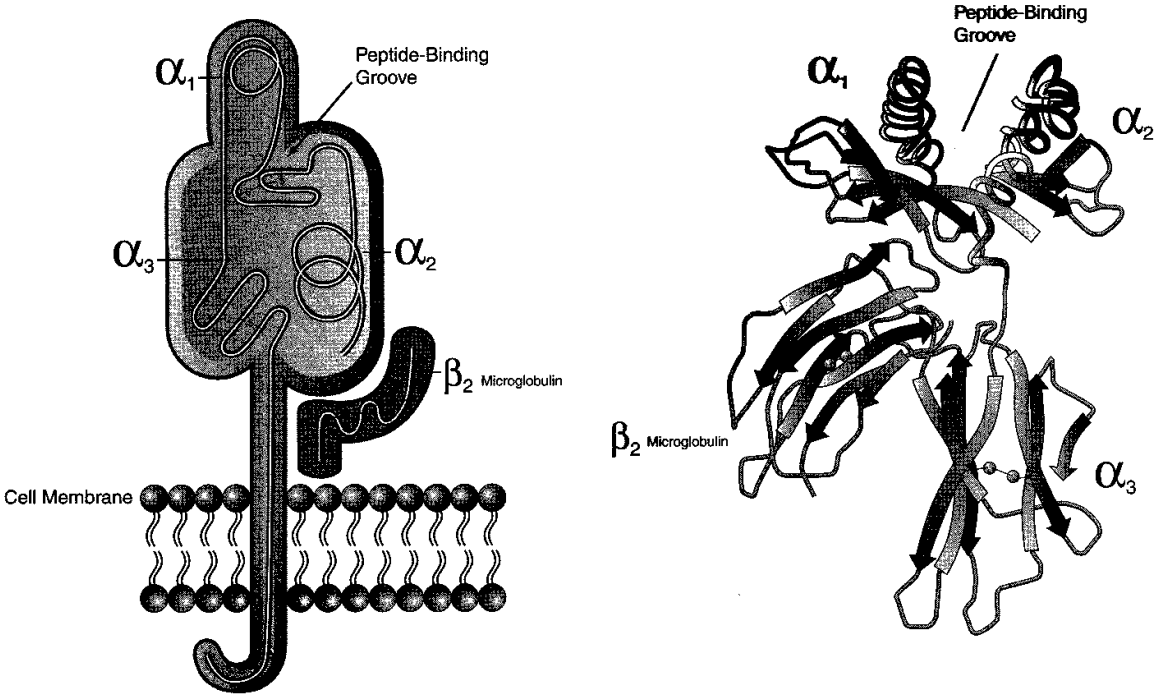


Figure II-3-3. ***Left)*** **The Class I MHC Molecule, and** ***right)*** **X-Ray Crystallographic Image of Class I MHC Peptide-Binding Groove**

In A Nutshell

Class II MHC
- α and β chains
- Expressed codominantly
- Present on APCs

Class II MHC molecules are expressed (also codominantly) on the antigen-presenting cells of the body (macrophages, B lymphocytes, dendritic cells, and Langerhans cells). The molecules are two chain structures of similar length, called α and β, and each possesses two extracellular domains and one intracytoplasmic domain. A groove that will accommodate peptides to be presented to the TCR is formed at the N-terminal end of both chains.

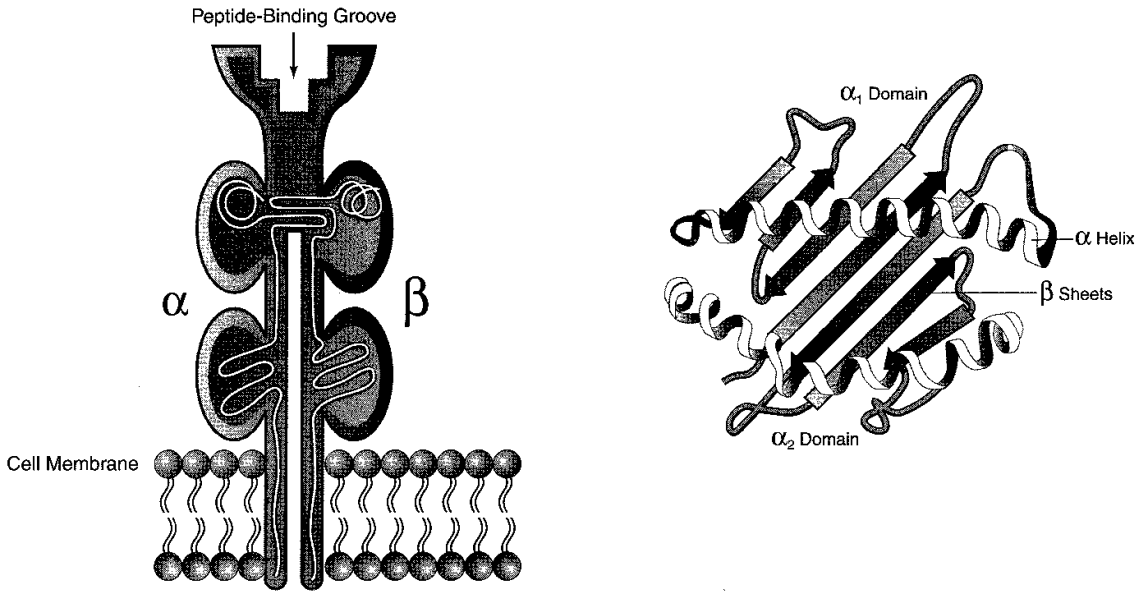


Figure II-3-4. ***Left)*** **The Class II MHC Molecule, and** ***right)*** **X-ray Crystallographic Image of Class II MHC Peptide-Binding Groove**

In A Nutshell

- Cells with "good" receptors receive positive selection.
- Cells with "useless" receptors receive no positive selection.
- Cells with "bad" receptors receive negative selection.
- CD4 stabilizes MHC II/TCR interaction.
- CD8 stabilizes MHC I/TCR interaction.

Within the thymus, each of these MHC products, loaded with normal self-peptides, is presented to the developing thymocytes. Those that have TCRs capable of binding with low affinity will receive a **positive selection** signal to divide and establish clones that will eventually mature in the medulla. Those that fail to recognize self-MHC at all will not be encouraged to mature (**failure of positive selection**). Those that bind too strongly to self MHC molecules will be induced to undergo apoptosis (**negative selection**) because these cells would have the potential to cause autoimmune disease. Although immature thymocytes express two accessory molecules on their surfaces designed to stabilize the interaction between MHC and TCR called **CD4** and **CD8**, as the affinity of the TCR for class I or class II MHC is "evaluated," the cells are directed to express only CD8 if their TCR binds class I molecules and only CD4 if their TCR binds class II molecules.

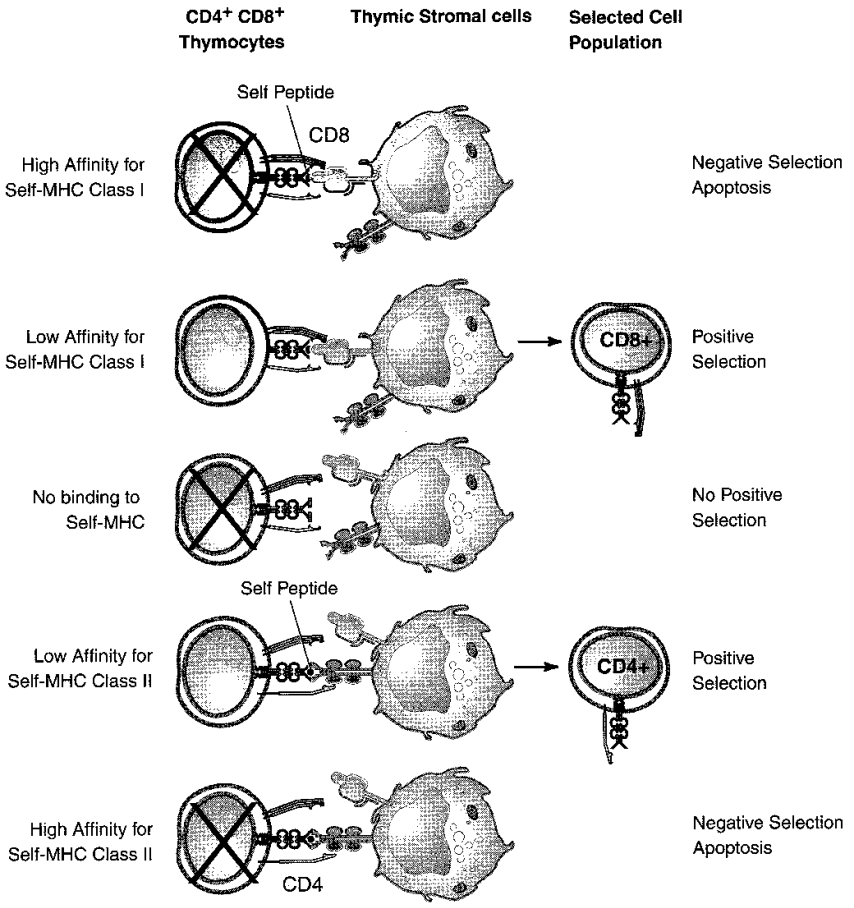


Figure II-3-5. T-Cell Selection in the Thymus

This selection process is an extraordinarily rigorous one. A total of 95 to 99% of all T-cell precursors entering the thymus are destined to die there. Only those with TCRs appropriate to protect the host from foreign invaders will be permitted to leave to the periphery: CD4+ cells that recognize class II MHC are destined to become **"helper" T cells** (TH), and CD8+ cells that recognize class I MHC are destined to become **cytotoxic T cells** (CTLs).

In A Nutshell

CD4+ cells that recognize class II MHC = TH cells.

CD8+ cells that recognize class I MHC = CTLs.

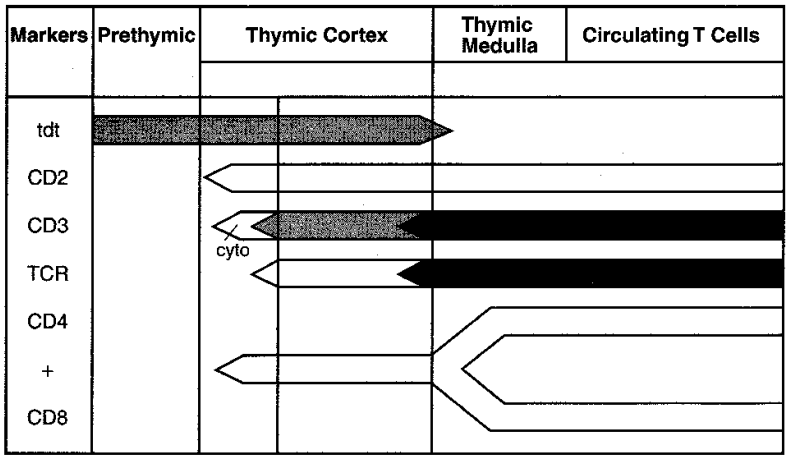


Figure II-3-6. Human T-Cell Differentiation

Chapter Summary

- The bone marrow and thymus are primary lymphoid organs in which the early development and selection of lymphocytes occurs (lymphopoiesis).
- Self-tolerance is induced by deletion of self-reactive cells in the bone marrow (clonal deletion) or inactivation of self-reactive cells in the periphery (clonal anergy).
- T-cell precursors move from the bone marrow to the thymus where they are selected for self-tolerance by exposure to major histocompatibility complex (MHC) antigens on stromal cells.
- Class I MHC products are two chain structures: the α chain is encoded within the MHC and β_2-microglobulin is not.
- Class I MHC products are expressed on all nucleated cells of the body in a codominant fashion.
- Class II MHC products are two chain structures of which both α and β chains are encoded within the MHC.
- Class II MHC products are expressed on antigen-presenting cells in a codominant fashion.
- Thymocytes with antigen receptors that bind self-peptides presented in the groove of MHC I or II molecules will be induced to undergo apoptosis (negative selection).
- Thymocytes with antigen receptors that have no binding affinity whatsoever for classes I or II MHC are not directed to mature further (failure of positive selection).
- Thymocytes with antigen receptors that can recognize "altered" self are encouraged to clone themselves and mature (positive selection) and express CD4 molecules if their affinity is for MHC class II. These will become helper T cells.
- Thymocytes with antigen receptors that can recognize "altered" self are encouraged to clone themselves and mature (positive selection) and express CD8 molecules if their affinity is for MHC class I. These will become cytotoxic T cells.

Review Questions

1. An 8-year-old boy is diagnosed with acute lymphoblastic leukemia. Flow cytometry is used to determine the immunophenotype of the malignant cells. The patient's cells are evaluated with monoclonal antibodies for MHC class II, CD19, and CD34, and are found to have high levels of fluorescence with all of these markers. They also possess cytoplasmic μ heavy chains. What is the developmental stage of these cells?

   (A) Immature B cell
   (B) Lymphoid progenitor cell
   (C) Mature B cell
   (D) Pre-B cell
   (E) Pro-B cell

2. The blood from an 8-year-old boy was analyzed by flow cytometry. The cells were treated with fluorescent-labeled antibodies to various cell surface markers before they were evaluated by flow cytometry. Which of the following markers would identify the B lymphocytes in the sample?

   (A) CD3
   (B) CD4
   (C) CD8
   (D) CD19
   (E) CD56

3. An 18-year-old member of a college soccer team is seen by a physician because of chest tightness and dyspnea on exertion. A 15-cm mediastinal mass is detected radiographically. Eighty percent of the white blood cells in the peripheral blood are small, abnormal lymphocytes with lobulated nuclei and scant cytoplasm. Immunophenotyping of the abnormal cells shows them to be CD4+ and CD8+. Where would such cells normally be found in the body?

   (A) Bone marrow
   (B) Peripheral blood
   (C) Thymic cortex
   (D) Thymic medulla
   (E) Splenic periarteriolar lymphoid sheaths

4. A 12-year-old child is diagnosed with a T-cell lymphoma. The phenotype of the malignant cell matches that of normal progenitor cells that leave the bone marrow to enter the thymus. What cell surface markers would you expect to find on the malignant cells?

   (A) CD4–, CD8–, TCR–
   (B) CD4–, CD8–, TCR+
   (C) CD4–, CD8+, TCR+
   (D) CD4+, CD8–, TCR+
   (E) CD4+, CD8+, TCR+

5. Herpes simplex viruses are extremely successful pathogens because they have a variety of immunologic evasion mechanisms. For example, both HSV I and II depress the expression of MHC class I molecules on the surface of infected cells. Which coreceptor's binding would be inhibited by this technique?

   (A) CD2
   (B) CD4
   (C) CD8
   (D) CD16
   (E) CD56

Answers

1. **The correct answer is D.** The leukemic cells are pre-B cells. They have rearranged their immunoglobulin genes to encode a μ heavy chain. MHC class II antigens are expressed beginning at the pro-B cell stage, as are CD19 and CD20. CD34 is a marker for early lymphohematopoietic stem and progenitor cells, and it functions as a cell–cell adhesion molecule. These cells would also have expressed CD10, the common acute lymphoblastic leukemia antigen (CALLA), which functions as a metalloendopeptidase.

   Immature B cells (**choice A**) have accomplished both and heavy and light immunoglobulin chain rearrangements and therefore express IgM molecules on their cell surface. They would be Tdt-negative, CD19- and CD20-positive, MHC class II-positive, and CD34-negative.

   Lymphoid progenitor cells (**choice B**) would not have completed any of the gene rearrangements necessary to create an immunoglobulin molecule. They would be Tdt-negative, MHC class II–negative, CD19- and CD20-negative, and CD34-positive.

   Mature B cells (**choice C**) possess surface IgM and IgD molecules and are capable of responding to foreign antigen. They are Tdt-negative, MHC class II-positive, CD19- and CD20-positive, CD34-negative, and may express CD40.

   Pro-B cells (**choice E**) are rearranging their immunoglobulin heavy chain gene segments but have not yet completed the process. Therefore, they have no completed chains either cytoplasmically or on their cell surfaces. They would be positive for Tdt, MHC class II, CD19, and CD20.

2. **The correct answer is D.** The best markers for identification of B lymphocytes are CD19, CD20, and CD21. CD19 and CD21 form a coreceptor complex during B-cell activation. The role of CD20 in B-cell activation is unclear, although it forms a calcium-ion channel. CD21 is also a receptor for the C3d component of complement and the Epstein-Barr virus.

   CD3 (**choice A**) is the signal transduction complex of T cells. It is found on all T cells in association with the T-cell antigen receptor.

   CD4 (**choice B**) is found on all helper T lymphocytes.

   CD8 (**choice C**) is found on all cytotoxic T lymphocytes.

   CD56 (**choice E**) is a marker for human natural killer cells.

3. **The correct answer is C.** This patient has a T-cell lymphoblastic lymphoma. In his case, the malignant cell is "double-positive": it possesses both CD4 and CD8. In a normal individual, these would only be found as an early developmental stage in the cortex of the thymus. Once cells have rearranged their receptor genes and been subjected to positive and negative selection, the cells leaving the thymus will express one coreceptor or the other but never both.

   Bone marrow (**choice A**) would contain T lymphocyte precursors that are double negative: They will lack both CD4 and CD8.

   Peripheral blood (**choice B**) would have mature T cells that have differentiated into either helper (CD4+) or cytotoxic (CD8+) cells. There should be no double-positive T cells in the peripheral blood.

   Thymic medulla (**choice D**) is the location of maturing T cells ready to circulate into the bloodstream and peripheral lymphoid organs. It would have only single-positive cells.

   Splenic periarteriolar lymphoid sheaths (**choice E**) are the T-cell–dependent areas of the spleen. They would have fully committed helper (CD4+) or cytotoxic (CD8+) cells.

4. **The correct answer is A.** T-lymphocyte precursors that leave the bone marrow and move to the thymus have neither CD4 nor CD8 coreceptors, and they have not rearranged the DNA of the variable domains of their antigen receptor, the TCR.

CD4–, CD8–, and TCR+ (**choice B**) is not a possible T-cell phenotype. Once the TCR gene segments are rearranged and the TCR is expressed, the cells will bear both CD4 and CD8 coreceptors.

CD4–, CD8+, and TCR+ (**choice C**) is the phenotype of cytotoxic T cells that would be in the circulation, not in the thymus, unless it were immediately prior to their release into the circulation following the thymic selection process.

CD4+, CD8–, and TCR+ (**choice D**) is the phenotype of helper T cells that would be in the circulation, not in the thymus, unless it were immediately prior to their release into the circulation following thymic selection processes.

CD4+, CD8+, and TCR+ (**choice E**) is the phenotype of cells in the thymic cortex. These are the cells that have rearranged their receptor genes and bear both CD4 and CD8 coreceptors. As the specificity of their TCR is tested, they will be directed to express either CD4 (and become a helper T cell) or CD8 (and become a cytotoxic T cell).

5. **The correct answer is C.** The interaction between the TCR and MHC class I/peptide conjugates is stabilized by the CD8 coreceptor. By downregulating the expression of MHC class I antigens on the surface of infected cells, the virus protects the infected host cell from killing by cytotoxic T lymphocytes.

   CD2 (**choice A**), also known as LFA-2, is a adhesion molecule within the immunoglobulin superfamily of genes. Its ligand is the integrin LFA-3. It is found on T cells and mediates attachment to other lymphocytes and antigen-presenting cells. It does not have a coreceptor role that would impact MHC class I–restricted killing.

   CD4 (**choice B**) is the coreceptor that stabilizes the interaction between MHC class II antigens and the TCR. It is thus important for helper T cells, not cytotoxic T cells.

   CD16 (**choice D**) is the Fc receptor involved in binding to immune complexes and promoting antibody-dependent cell-mediated cytotoxicity. It is not involved in the MHC class I–restricted killing by cytotoxic T cells.

   CD56 (**choice E**) is a cell surface marker found on NK cells. Its function is unknown. However, since NK activity is enhanced in the absence of MHC class I antigen expression, the downregulation of these molecules by herpes simplex I and II actually makes infected cells more susceptible to the NK cell form of lysis.

Lymphocyte Recirculation and Homing

4

What The USMLE Requires You To Know

- The structure and function of the secondary lymphoid organs
- The areas in which B and T lymphocytes localize in the peripheral lymphoid organs
- The role of chemokines and adhesion molecules in lymphocyte trafficking

Lymphocytes of the B- and T-cell lineages that have completed their selection in the bone marrow and thymus respectively are now **mature, naive lymphocytes** ready to begin their role in the surveillance of the body against invaders. These mature, naive lymphocytes will begin the process of **recirculation** through the body, which is essential for ensuring that the limited number of cells with receptors for a specific antigen is enabled to search for that antigen throughout the body. Naive cells preferentially recirculate through the **peripheral (secondary) lymphoid organs**, the **lymph nodes, spleen, and mucosal-associated lymphoid tissue (MALT)** to maximize the chances of encounter with foreign antigen and thereby initiate specific immune responses.

Lymph nodes are the small nodular aggregates of secondary lymphoid tissue found along the lymphatic channels of the body and are designed to initiate immune responses to **tissue-borne antigens.** Each lymph node is surrounded by a fibrous capsule that is punctured by **afferent lymphatics,** which bring lymph into the **subcapsular sinus.** The fluid percolates through an outer **cortex** area that contains aggregates of cells called **follicles.** The lymph then passes into the inner **medulla** and the **medullary sinus** before leaving the node through the **hilum** in an efferent lymphatic vessel. Ultimately, lymph from throughout the body is collected into the **thoracic duct,** which empties into the vena cava and returns it to the blood.

In A Nutshell

Peripheral (Secondary) Lymphoid organs

- Lymph nodes, spleen, and MALT
- Sites of foreign antigen exposure

In A Nutshell

- Lymph nodes filter tissue fluids.
- Outer cortex contains follicles (B-cell areas).
- Paracortex is a T-cell area.
- Inner medulla contains macrophages.

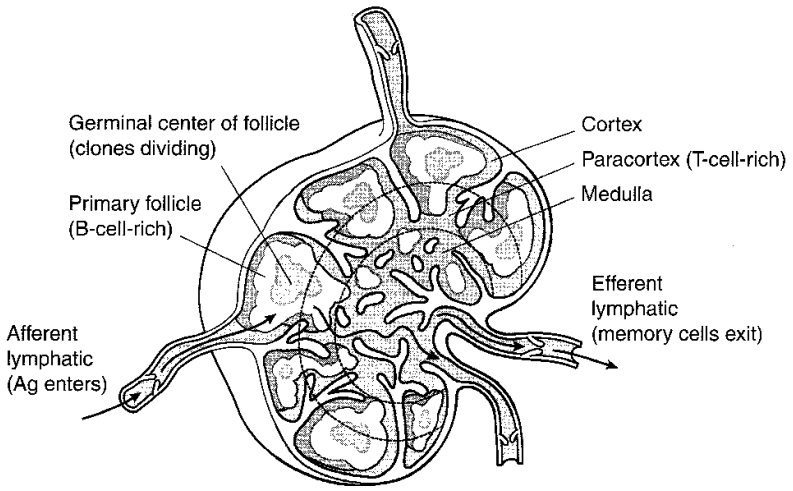


Figure II-4-1. Compartmentalization of a Lymph Node

In A Nutshell

- The spleen filters blood.
- PALS are T-cell areas.
- Follicles are B-cell areas.

The **spleen** is the secondary lymphoid organ designed to initiate immune responses to **blood-borne antigens.** A single splenic artery enters the capsule at the hilum and branches into arterioles, which become surrounded by cuffs of lymphocytes, the **periarteriolar lymphoid sheaths** (PALS). **Lymphoid follicles** surrounded by a rim of lymphocytes and macrophages are attached nearby. This constitutes the **white pulp**. The arterioles ultimately end in vascular **sinusoids,** which make up the **red pulp**. From here, venules collect blood into the splenic vein, which empties into the portal circulation.

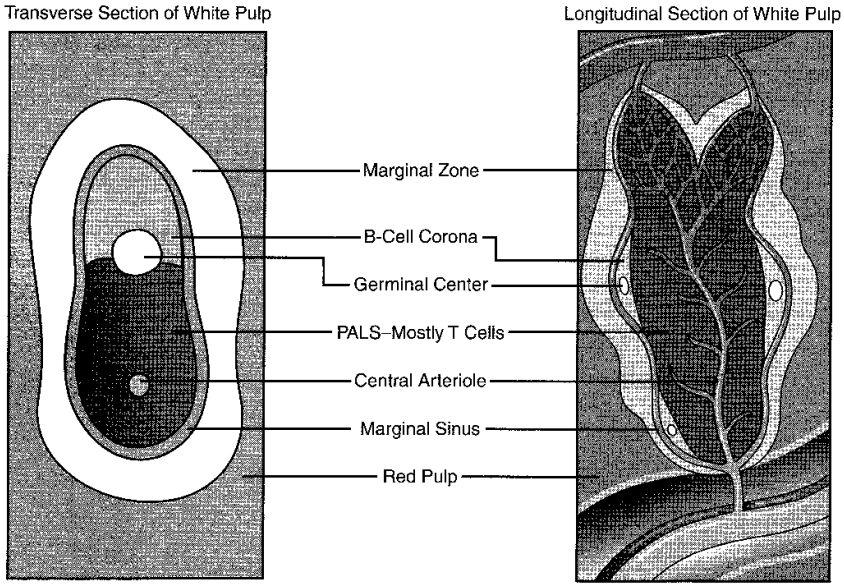


Figure II-4-2. The Structure of the Spleen

Naive lymphocytes enter the lymph nodes from the blood through high endothelial venules (HEVs). This migration involves a multistep sequence of interactions between the lymphocyte and adhesion molecules on the endothelium. The initial low-affinity interaction is mediated by homing receptors, called **L-selectins**, present on the lymphocytes, which bind to addressins present on the HEVs.

Naive T cells migrate into the **paracortical** areas under the influence of a chemokine gradient. Naive B cells are guided along a chemokine gradient into the **lymphoid follicles**.

Less is known about lymphocyte recirculation through the spleen. Naive T cells home to the **periarteriolar lymphoid sheaths** (PALS), whereas naive B cells home to the lymphoid follicles.

The rate of recirculation of naive lymphocytes through the body by this system is quite high. It is estimated that each lymphocyte passes through each lymph node in the body once a day and through the spleen once every two days on average. This high rate of trafficking maximizes the possibility that the very small number of cells having a specific antigen receptor have the maximum possibility of encountering that antigen when it is present.

Chapter Summary

- Mature naive lymphocytes recirculate through the peripheral lymphoid organs (lymph nodes and spleen) to search for antigen.
- Lymph nodes are designed to filter antigens from the tissue fluids.
- Lymph enters through afferent lymphatics and percolates through an outer cortex and inner medulla before leaving through the efferent lymphatic in the hilus.
- The spleen is designed to filter antigens from blood. Blood enters through a single splenic artery, which branches into arterioles that become surrounded by cuffs of lymphocytes (periarteriolar lymphoid sheaths) with follicles and macrophages nearby.
- Lymphocytes leave the bloodstream to enter lymph nodes through high endothelial venules.
- L-selectins on lymphocytes bind to addressins on the high endothelial venules, and chemokine receptors mediate the homing of specific cells to specific areas.
- T lymphocytes migrate into the lymph node paracortical areas.
- B lymphocytes migrate into the lymph node follicles.
- In the spleen, T lymphocytes are attracted to the periarteriolar lymphoid sheaths.
- B cells entering the spleen are attracted to the lymphoid follicles.
- Every lymphocyte passes through every lymph node once per day and through the spleen once every two days on average.

In A Nutshell

- Naive lymphocytes leave the blood through HEVs.
- L-selectins on lymphocytes bind to addressins on HEVs.
- Naive T cells home to the lymph node paracortex or the splenic PALS.
- Naive B cells home to lymphoid follicles.

Review Questions

1. A lymph node biopsy of a 6-year-old boy shows markedly decreased numbers of lymphocytes in the paracortical areas. Analysis of his peripheral blood leukocytes is likely to show normal to elevated numbers of cells expressing surface

   (A) CD2
   (B) CD3
   (C) CD4
   (D) CD8
   (E) CD19

2. A 65-year-old woman was involved in an automobile accident that necessitated the removal of her spleen. To which of the following pathogens would she have the most increased susceptibility?

   (A) *Babesia microti*
   (B) *Bordetella pertussis*
   (C) *Corynebacterium diphtheriae*
   (D) Enteroaggregative *Escherichia coli*
   (E) Human papilloma virus

3. A 4-year-old boy is referred to a specialist for the diagnosis of a possible immunologic problem. The child has extremely elevated white blood cell counts, with a profound lymphocytosis. A biopsy performed on a cervical lymph node reveals extreme hypocellularity in both cortical and paracortical areas. Absence of which of the following leukocyte surface molecules could result in this clinical picture?

   (A) Addressins
   (B) Chemokines
   (C) Immunoglobulin family cell adhesion molecules
   (D) Integrins
   (E) L-selectins

4. A 6-year-old child is taken to his pediatrician because the parents are alarmed about an indurated fluctuant mass on the posterior aspect of his neck. The mass is nontender and shows no signs of inflammation. The child is examined carefully, and no other masses are found. The pediatrician decides to submit a biopsy of this area to a pathologist. The pathologist reports back that the mass is a lymph node with markedly increased numbers of cells in the cortical area. Fluorescent antisera to which of the cell surface markers is most likely to bind to cells in this area?

   (A) CD2
   (B) CD3
   (C) CD4
   (D) CD16
   (E) CD19

5. A radioactive tracer dye is injected subcutaneously into the forearm of an experimental subject. What is the first area of the first draining lymph node that would develop significant radioactivity?

   (A) Cortex
   (B) Medulla
   (C) Paracortex
   (D) Primary follicle
   (E) Subcapsular sinus

Answers and Explanations

1. **The correct answer is E.** The paracortex of a lymph node is a T-cell–dependent area. If this area is lacking cellularity, then the patient has a deficiency of T lymphocytes. B-lymphocyte numbers could be normal or even elevated. The only B-cell marker on this list is CD19, the marker which is used clinically to enumerate B cells in the body.

   CD2 (**choice A**), also known as LFA-2, is an adhesion molecule found on T cells, thymocytes, and NK cells. In a person with a T-cell deficiency, there would be decreased numbers of cells bearing this marker.

   CD3 (**choice B**) is found on all T cells. It is also called the "pan-T" cell marker. In a person with a T-cell deficiency, there would be decreased numbers of cells bearing this marker.

   CD4 (**choice C**) is found on all helper T lymphocytes. In a person with a T-cell deficiency, there would be decreased numbers of cells bearing this marker.

   CD8 (**choice D**) is found on all cytotoxic T lymphocytes. In a person with a T-cell deficiency, there would be decreased numbers of cells bearing this marker.

2. **The correct answer is A.** The spleen is the secondary lymphoid organ that is responsible for primary surveillance against blood-borne antigens. *Babesia microti* is an intraerythrocytic parasite of humans, transmitted by the same vector tick as Lyme disease. Red blood cells (and their parasites) are filtered by the spleen, so splenectomy is a predisposing factor in development of serious disease with this parasite.

   Bordetella pertussis (**choice B**) is a mucosal surface pathogen that attaches to the upper airways. Although its toxin becomes blood-borne, the organism itself is confined to the respiratory tree.

   Corynebacterium diphtheriae (**choice C**) is a mucosal surface pathogen that attaches to the upper airways. Although its toxin becomes blood-borne, the organism itself is confined to the respiratory tree.

   Enteroaggregative *Escherichia coli* (**choice D**) is an organism that causes diarrhea by producing a biofilm-like aggregation of organisms on the surface of the colonic mucosa, which impedes absorption. It is not likely to be a blood-borne pathogen.

   Human papilloma virus (**choice E**) produces localized infections in epithelial cells where it is transferred by human-to-human or human-to-fomite contact. It is not likely to be a blood-borne pathogen.

3. **The correct answer is E.** L-selectins are the molecules found on the surfaces of lymphocytes that mediate their binding to the high endothelial venules of lymph nodes. This is the means by which lymphocytes enter lymph nodes; without these molecules, they would be unable to leave the blood. Thus, they would rise in the blood to extreme levels and be absent from their appropriate areas in the secondary lymphoid organs.

   Addressins (**choice A**) are the molecules complementary to L-selectins, which are found on endothelial cells. Although their absence could also cause similar signs, the question asks about a leukocyte surface molecule, and addressins are not found on leukocytes.

   Chemokines (**choice B**) are not cell surface molecules, but do play a role in the homing of lymphocytes to specific regions of the secondary lymphoid organs. An absence of chemokine receptors on leukocytes could have caused similar signs, but chemokines themselves are soluble substances, not surface molecules.

   Immunoglobulin family cell adhesion molecules (**choice C**), or IgCAMs, are adhesion molecules found on various cells of the immune system. They mediate cell-to-cell interactions, as well as binding to the extracellular matrix. Their ligands are the integrins. If these molecules were absent, cell-cell interactions would be diminished, but movement of lymphocytes out of the circulation and into the secondary lymphoid organs would not be affected.

Integrins (**choice D**) are the ligands for the IgCAMs. They are involved in cell-to-cell interactions, as well as binding to the extracellular matrix. If these molecules were absent, cell-cell interactions would be diminished, but movement of lymphocytes out of the circulation and into the secondary lymphoid organs would not be affected.

4. **The correct answer is E.** The cortex of lymph nodes is a B-lymphocyte area. Thus, cells in this area would stain with fluorescent antibodies against CD19, the molecule that serves as a portion of the B-cell signal transduction complex. This molecule would be found on all B cells, but would be absent from T cells, macrophages, and NK cells.

   CD2 (**choice A**) is a T-cell marker. T cells will be found in the paracortical areas of lymph nodes.

   CD3 (**choice B**) is a T-cell marker. It is the signal transduction complex of the T cell and will be found on all T cells. T cells will be found in the paracortical areas of lymph nodes.

   CD4 (**choice C**) is a marker for helper T cells. These cells would be found in the paracortical areas of lymph nodes.

   CD16 (**choice D**) is the Fc receptor for IgG antibodies. It would be found on natural killer cells, which would not be numerous in the cortex of the lymph nodes.

5. **The correct answer is E.** Lymph nodes are designed to filter tissue fluids. Fluids entering the lymph nodes do so through the afferent lymphatics and are released into the subcapsular sinus. From there, fluids percolate through the cortex, into the medulla, through the medullary cords, and finally exit through the efferent lymphatics in the hilum.

   The cortex (**choice A**) of the lymph node is directly beneath the subcapsular sinus. It would be the second region of the lymph node to be exposed to the radioactive tracer. The cortex is a B-lymphocyte–rich area.

   The medulla (**choice B**) of the lymph node is rich in macrophages. It would not receive the radioactive fluid until it had passed through the cortex and paracortex.

   The paracortex (**choice C**) of the lymph node is a T-cell area. It lies between the cortex and the medulla and thus would receive the radioactive fluid after the cortical areas.

   Primary follicles (**choice D**) are found in the cortex of the lymph node. These are areas of active B-lymphocyte proliferation and cloning. They would receive the radioactivity after it left the subcapsular sinus.

The First Response to Antigen

5

What the USMLE Requires You to Know

- The meaning of antigen, epitope, antigenic determinant, and hapten
- The sequence of inflammatory events and their role in the initial response to invasion
- The mechanism and chemoattractive molecules involved in phagocyte extravasation
- The steps of phagocytosis and mechanisms of intracellular killing
- The meaning of opsonization and the molecules involved
- The clinical sequelae of defects in chemotaxis and phagocytosis (LAD and CGD)

Historically, the word **antigen** was meant to designate a substance capable of inducing the formation of a specific antibody: an **anti**body-**gen**erating substance. Today the word is used almost interchangeably with the word **immunogen**—an **immune** response–**gen**erating substance—to encompass any substance capable of activating and generating a response from any committed lymphocyte. For a molecule to be an immunogen, it needs to fit three basic criteria:

- It must be recognized as **foreign**.
- It must have a certain degree of **chemical complexity**.
- It must have a **molecular weight** of at least 5,000 to 10,000 K.

B lymphocytes are capable of recognizing molecules of almost any chemical composition. The portion of the foreign molecule that actually fits into the idiotype of the B-cell receptor is quite small: 5 to 6 amino acids in the case of a protein or 4 to 5 hexose units in the case of a carbohydrate. This portion of the molecule that has three-dimensional complementarity with the idiotype is called the **epitope** or **antigenic determinant** of the immunogen. Because most of the microbes that challenge the immune system are quite large, most naturally occurring antigens have many copies of the same epitopes. The size of a molecule is a critical factor in its immunogenicity because B lymphocytes can only be activated when their antigen receptors are **crosslinked** by the accommodation of more than one identical epitope. This serves as the first signal to then send the message through the signal transduction complex to activate the cell. Molecules that possess only one epitope are called **haptens** because they can occupy only one idiotype of the double-armed B-cell receptor. T lymphocytes recognize peptides of 10 to 20 amino acids in length only when presented to them in the groove of an MHC molecule on the surface of an antigen-presenting cell.

In A Nutshell

- An antigen or immunogen is a substance that can elicit an immune response.
- It must be foreign, chemically complex, and large.

Note

The idiotype of a cell receptor fits the epitope (antigenic determinant) of the antigen.

Note

Haptens are single antigenic determinants.

Clinical Correlate

Drug allergies with penicillin and other agents such as streptomycin, aspirin, sulfa drugs, succinyl choline, and some opiates can be induced by small doses of the drug and are not consequences of the pharmacologic or physiologic effects of the drugs. Typically, an allergic response occurs 7 to 14 days following exposure, and the first symptoms may be mild. Subsequent drug exposures can result in severe and life-threatening anaphylaxis (see Chapter 13). Most drugs are low molecular weight compounds that are not capable of inducing immune responses by themselves—they act as **haptens**. Inside the body, however, these agents can become conjugated to body proteins (the **carrier**), and the hapten-carrier conjugate serves as the immunogen for the ensuing allergic response.

In A Nutshell

- The acute inflammatory response is a first response to invasion.
- Cytokines increase expression of selectin-type adhesion molecules.

Antigens are normally introduced into the body across the mucosa or the epithelia. The **acute inflammatory response** is often the first response to this invasion and represents a response of the innate immune system to block the challenge. The first step in the acute inflammatory response is activation of the vascular endothelium in the breached epithelial barrier. **Cytokines** and other inflammatory mediators released in the area as a result of tissue damage induce expression of **selectin-type adhesion molecules** on the endothelial cells. **Neutrophils** are usually the first cell to bind to the inflamed endothelium and extravasate into the tissues, peaking within 6 hours. Monocytes, macrophages, and even eosinophils may arrive 5 to 6 hours later in response to neutrophil-released mediators.

In A Nutshell

Extravasation of phagocytes involves rolling, activation by chemoattractants, arrest/adhesion, and transendothelial migration.

The extravasation of phagocytes into the area requires four sequential, overlapping steps:

Step 1: Rolling

Phagocytes attach loosely to the endothelium by low-affinity, selectin-carbohydrate interactions. E-selectin molecules on the endothelium bind to mucin-like adhesion molecules on the phagocyte membrane and bind the cell briefly, but the force of blood flow into the area causes the cell to detach and reattach repeatedly, rolling along the endothelial surface until stronger binding forces can be elicited.

Step 2: Activation by chemoattractants

Chemokines released in the area during inflammation, such as interleukin 8 (IL-8), complement split product C5a, and N-formyl peptides produced by bacteria bind to receptors on the phagocyte surface and trigger a G-protein–mediated activating signal. This signal induces a conformational change in integrin molecules in the phagocyte membrane that increases their affinity for immunoglobulin-superfamily adhesion molecules on the endothelium.

Step 3: Arrest and adhesion

Interaction between **integrins** and **Ig-superfamily cellular adhesion molecules** (Ig-CAMs) stabilizes adhesion of the phagocyte to the endothelial cell.

Step 4: Transendothelial migration

The phagocyte extends pseudopodia through the vessel wall and extravasates into the tissues.

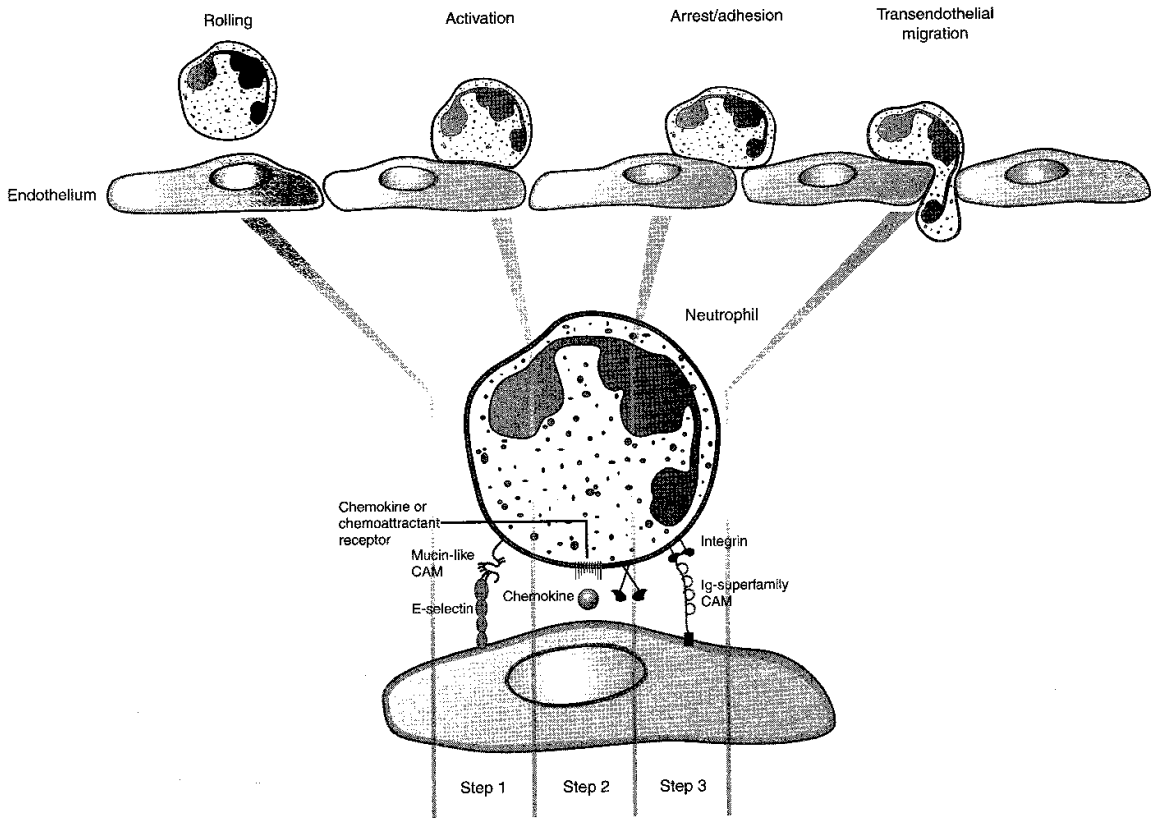


Figure II-5-1. The Steps of Phagocyte Extravasation

Clinical Correlate

Leukocyte adhesion deficiency is a rare autosomal recessive disease in which there is an absence of **CD18**, which is the common β_2 chain of a number of integrin molecules. A key element in the migration of leukocytes is integrin-mediated cell adhesion, and these patients suffer from an inability of their leukocytes to undergo adhesion-dependent migration into sites of inflammation. The first indication of this defect is often omphalitis, a swelling and reddening around the stalk of the umbilical cord. These patients are no more susceptible to virus infection than are normal controls, but they suffer recurrent, chronic bacterial infections. These patients frequently have abnormally high numbers of granulocytes in their circulation, but migrations into sites of infection is not possible, so **abscess and pus formation do not occur.**

One method of diagnosing LAD involves evaluating expression (or lack thereof) of the β chain (CD18) of the integrin by flow cytometry.

Bacterial infections in these patients can be treated with antibiotics, but they recur. If a suitable bone marrow donor can be found, the hematopoietic system of the patient is destroyed with cytotoxic chemicals and a bone marrow transplant is performed.

Once in the tissues, neutrophils express increased levels of receptors for chemoattractants and exhibit **chemotaxis** migrating up a concentration gradient toward the attractant. Neutrophils release chemoattractive factors that call in other phagocytes.

In A Nutshell

Substances chemoattractive to neutrophils include IL-8, C5a, fibrinopeptides, leukotriene B_4, and formyl methionyl peptides.

Table II-5-1. Chemoattractive Molecules

Chemoattractive Molecule	Origin
Chemokines (IL-8)	Tissue mast cells, platelets, neutrophils, monocytes, macrophages, eosinophils, basophils, lymphocytes
Complement split product C5a	Endothelial damage → activation Hageman factor → plasmin activation
Fibrinopeptides	Endothelial damage → activation Hageman factor → thrombin → fibrin clot degradation
Leukotriene B_4	Membrane phospholipids of macrophages, monocytes, neutrophils, mast cells → arachidonic acid cascade → lipoxygenase pathway
Formyl methionyl peptides	Released from microorganisms

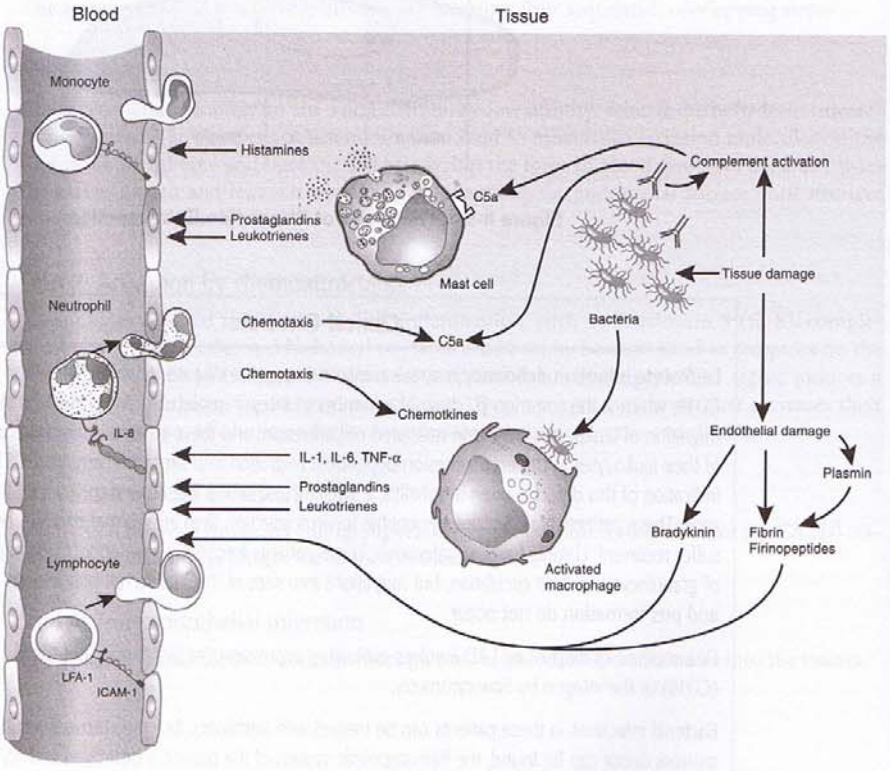


Figure II-5-2. The Acute Inflammatory Response

Once chemotaxis of phagocytic cells into the area of antigen entry is accomplished, these cells ingest and digest particulate debris, such as microorganisms, host cellular debris, and activated clotting factors. This process, called **phagocytosis**, involves:

- Extension of pseudopodia to engulf attached material
- Fusion of the pseudopodia to trap the material in a phagosome
- Fusion of the phagosome with a lysosome to create a phagolysosome
- Digestion
- Exocytosis of digested contents

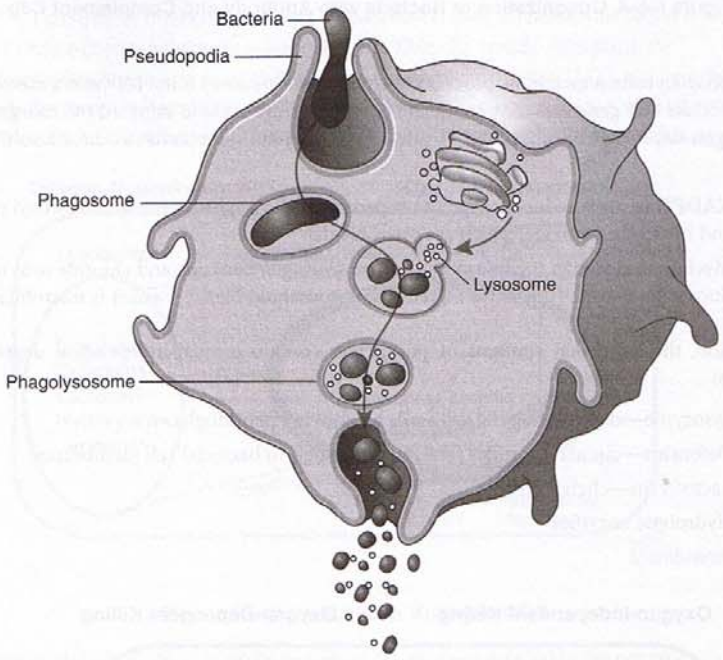


Figure II-5-3. Phagocytosis

Both macrophages and neutrophils have membrane receptors for certain types of antibody (**IgG**) and certain complement components (**C3b**). If an antigen is coated with either of these materials, adherence and phagocytosis may be enhanced by up to 4,000-fold. Thus, antibody and complement are called **opsonins**, and the means by which they enhance phagocytosis is called **opsonization**.

In A Nutshell

Phagocytosis involves:

- Extension of pseudopodia
- Formation of phagosome
- Fusion with lysosome to form phagolysosome
- Digestion
- Exocytosis

In A Nutshell

Opsonization is enhancement of phagocytosis with:

- IgG
- C3b

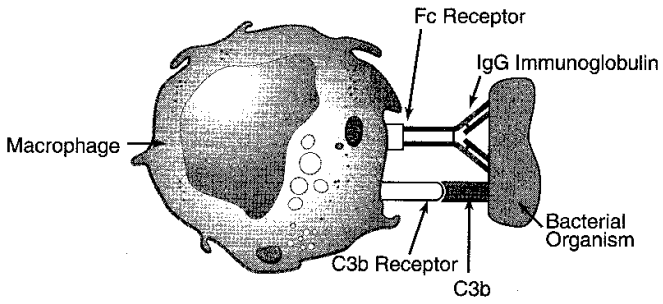


Figure II-5-4. Opsonization of Bacteria with Antibody and Complement C3b

In A Nutshell

Intracellular killing mechanisms include:

- Toxic oxygen metabolites
- Toxic halide radicals
- Lysosomal contents:
  - Lysozyme
  - Defensins
  - Lactoferrin
  - Hydrolytic enzymes

During phagocytosis, a metabolic process known as the respiratory burst activates a membrane-bound oxidase that generates oxygen metabolites, which are toxic to ingested microorganisms. Two oxygen-dependent mechanisms of intracellular digestion are activated as a result of this process.

- **NADPH oxidase** reduces oxygen to superoxide anion, which generates hydroxyl radical and hydrogen peroxide, which are microbicidal.
- **Myeloperoxidase** in the lysosomes acts on hydrogen peroxide and chloride ions to produce hypochlorite (the active ingredient in household bleach), which is microbicidal.

In addition, the lysosomal contents of phagocytes contain oxygen-independent degradative materials:

- Lysozyme—digests bacterial cell walls by cleaving peptidoglycan
- Defensins—circular peptides that form channels in bacterial cell membranes
- Lactoferrin—chelates iron
- Hydrolytic enzymes

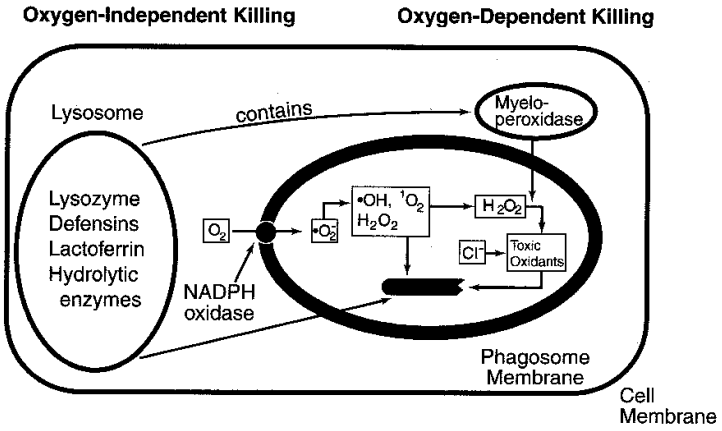


Figure II-5-5. Metabolic Stimulation and Killing Within the Phagocyte

Clinical Correlate

Chronic Granulomatous Disease

When defects occur in the ability of phagocytes to perform their critical functions as first responders and intracellular destroyers of invading antigens, clinically important pathologic processes ensue. Such defects tend to make the patient susceptible to severe infections with **extracellular bacteria and fungi**.

Chronic granulomatous disease (CGD) is an inherited deficiency in the production of one of several subunits of **NADPH oxidase**. This defect eliminates the phagocyte's ability to produce many critical oxygen-dependent intracellular metabolites ($\cdot O_2^-$, $\cdot OH$, $^1O_2$, and H_2O_2). The two other intracellular killing mechanisms remain intact (myeloperoxidase + $H_2O_2 \rightarrow$ HOCl and lysosomal contents). If the patient is infected with a catalase-negative organism, the H_2O_2 waste product produced by the bacterium can be used as a substrate for myeloperoxidase, and the bacterium is killed. If, however, the person is infected with a catalase-positive organism (e.g., *Staphylococcus, Klebsiella, Serratia, Aspergillus*), the myeloperoxidase system lacks its substrate (because these organisms destroy H_2O_2), and the patient is left with the oxygen-independent lysosomal mechanisms that prove inadequate to control rampant infections. Thus, CGD patients suffer from chronic, recurrent infections with **catalase-positive** organisms.

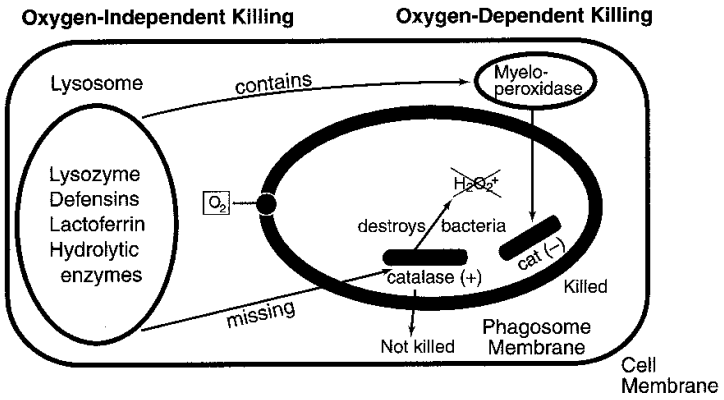


Figure II-5-6. Intracellular Killing in CGD

Failures of phagocytic cells to generate oxygen radicals are easily detected by the nitroblue tetrazolium (NBT) reduction test or neutrophil oxidative index (NOI; a flow cytometric assay).

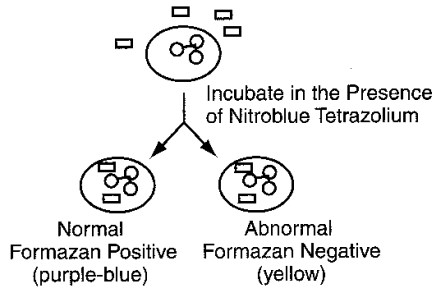


Figure II-5-7. Nitroblue Tetrazolium Reduction

Chapter Summary

- An antigen or immunogen is a substance capable of activating and generating a response from any committed lymphocyte.
- To be immunogenic, a substance must be recognized as foreign and have chemical complexity and sufficient size.
- The portion of an antigen that fits into the idiotype of an antigen receptor is called the epitope or antigenic determinant.
- Haptens are single antigenic determinants that can only generate immune responses if they are linked to larger carrier proteins. This is the mechanism of elicitation of many drug allergies.
- After antigen is introduced across an anatomic barrier, phagocytes (first neutrophils and then monocytes) will extravasate into the area of injury by *1*) rolling, *2*) activation with chemoattractants, *3*) arrest/adhesion, and *4*) transendothelial migration.
- Important chemoattractants include IL-8, C5a, leukotriene B_4, and N-formyl peptides.
- Integrins on the phagocyte membrane bind to Ig-CAMs on endothelia to mediate adhesion.
- Once through the endothelium, phagocytes are attracted to the area of injury by chemokines of the IL-8 family, complement split products, fibrinopeptides, leukotriene B_4, and formyl methionyl peptides.
- Phagocytosis involves: the formation of pseudopodia that trap particulate material in a phagosome; addition of lysosomes to cause intracellular digestion within the phagolysosome; and exocytosis of the digested materials.
- Opsonization is the coating of particles with IgG or C3b (or both) to enhance phagocytosis.
- Ingestion is accompanied by a respiratory burst, which generates the toxic metabolites necessary to destroy intracellular materials.
- NADPH oxidase produces toxic oxygen radicals, and myeloperoxidase generates hypochlorite.
- Lysosomal contents (lysozyme, defensins, lactoferrin, and hydrolytic enzymes) are also toxic to ingested material.
- Patients with chronic granulomatous disease (CGD; an inherited deficiency of NADPH oxidase) are susceptible to chronic, recurrent infections with catalase-positive organisms.

Review Questions

1. A rabbit hunter in Arkansas is diagnosed with ulceroglandular tularemia and treated with streptomycin. Within a week, he returns to the hospital. The tularemic papule, lymphadenopathy, and bacteremia have resolved, but he has now developed a raised, itching skin rash and a fever. The drug was discontinued, and the symptoms subsided. What was the role of streptomycin in this case?

   (A) It acted as a B-cell mitogen
   (B) It acted as a hapten
   (C) It acted as a provider of costimulatory signals
   (D) It acted as a superantigen
   (E) It acted as an immunogen

2. During World War II, when quinine was used as a prophylactic against malaria infections in U.S. personnel on long-term assignment to the South Pacific, a small proportion of soldiers developed blackwater fever (chronic kidney damage from the autoimmune effects of complement-mediated hemolysis of quininized red blood cells). In this case, the quinine played the role of

   (A) autoantigen
   (B) carrier
   (C) hapten
   (D) immunogen
   (E) reagin

3. A two-year-old child who has suffered recurrent bacterial infections is evaluated for immunologic deficiency. The child has age-normal numbers of CD19+ and CD3+ cells in the peripheral blood and an extreme neutrophilia. The nitroblue tetrazolium dye reduction test is normal. What is the most likely defect in this child?

   (A) Absence of CCR4
   (B) Absence of CD18
   (C) Absence of interleukin-1
   (D) Absence of interleukin-4
   (E) Absence of tumor necrosis factor-α

4. A 2-year-old boy is admitted to the hospital for workup of a possible immunologic disorder. His history is remarkable for the occurrence of multiple skin infections involving *Staphylococcus, Pseudomonas,* and *Candida.* On examination the child has cervical lymphadenopathy and mild hepatosplenomegaly. Blood tests reveal an elevated erythrocyte sedimentation rate and neutrophilia. The nitroblue tetrazolium dye reduction test and neutrophil oxidative index are negative. What is the most likely defect in this child?

   (A) C3 deficiency
   (B) Deficiency of CD18
   (C) Deficiency of myeloperoxidase
   (D) NADPH oxidase deficiency
   (E) Phagocyte granule structural defect

5. It has been learned in animal experiments that there are advantages to eliciting nonspecific inflammation at the site of inoculation of antigen toward the ultimate development of a protective immune response to that immunogen. Which of the following substances, if introduced with a vaccine, would serve the purpose of attracting a neutrophilic infiltrate into the area?

   (A) Complement component 3b
   (B) Immunoglobulin G
   (C) Interleukin-8
   (D) Myeloperoxidase
   (E) Tumor necrosis factor-α

Answers and Explanations

1. **The correct answer is B.** Many drug allergies, such as the one described here, are hapten-carrier immune responses. The drug is not large enough by itself to elicit an immune response (it is a hapten), but when it becomes covalently coupled to the body's own proteins (which act as carriers), the combined molecule becomes immunogenic, and a response against one's own tissues is elicited.

   Acting as a B-cell mitogen (**choice A**) is not correct. B-cell mitogens, such as pokeweed mitogen and lipopolysaccharide, cause polyclonal proliferation of B cells and elaboration of IgM antibodies. The drug allergy described here is not a polyclonal response, but a specific anti–altered-self response generated by T and B lymphocytes and production of IgE antibodies.

   Acting as a provider of costimulatory signals (**choice C**) is not correct. The costimulatory signals required to activate B and T lymphocytes include CD28/B7 and CD40/CD40L interactions. These are additional interactions (beyond the specific recognition of antigen) required for the activation of B and T lymphocytes. Although these costimulatory signals would be involved in the evolution of this allergic response, the streptomycin does not serve as a costimulatory signal.

   Acting as a superantigen (**choice D**) is not correct. Superantigens are materials that crosslink the variable β domain of the T-cell receptor and the α-chain of class II MHC molecules. They induce activation of all T cells that express receptors with a particular Vβ domain. The resulting T-cell mitogenesis causes overproduction of T-cell and macrophage cytokines and system-wide pathology. Toxic shock syndrome toxin-1 and *Streptococcus pyogenes* erythrogenic exotoxins act as superantigens.

   Acting as an immunogen (**choice E**) is not correct because streptomycin is not large enough to be immunogenic. Immunogens must be large enough to have at least two epitopes. It is only through binding to a larger carrier protein (the patient's own tissue proteins) that a hapten such as a drug can become immunogenic.

2. **The correct answer is C.** This is a case of autoimmune hemolytic anemia generated during a hapten-carrier immune response. The drug is not large enough by itself to elicit an immune response (it is a hapten), but when it becomes covalently coupled to the red blood cell proteins (which act as carriers), the combined molecule becomes immunogenic. Antibodies are generated and bind to the drug–RBC complex, complement is activated, and the red blood cells are lysed.

   Autoantigen (**choice A**) is not correct because the immune response is being generated against an "altered-self" component. The RBCs of the patient are not themselves the immunogen: It is the complex of drug–RBC protein that elicits the response. The effect is certainly similar to an autoimmune response: The patient destroys his own tissues. However, the eliciting immunogen is altered-self, so this is not a case where failure of self-tolerance causes disease.

   Carrier (**choice B**) is not correct because carriers must be protein molecules. The carrier component of a hapten-carrier complex is recognized by T lymphocytes. Because T lymphocytes are only capable of recognizing peptides presented in the groove of major histocompatibility complex antigens, a molecule such as quinine is "invisible" to T cells.

   Immunogen (**choice D**) is not correct because quinine is not large enough to be immunogenic. Immunogens must be large enough to have at least two epitopes. It is only through binding to a larger carrier protein (the patient's own tissue proteins) that a hapten such as a drug can become immunogenic.

   Reagin (**choice E**) is a word that is used to denote an immunogen that elicits an IgE-antibody response. Although some drug allergies are indeed IgE-mediated immediate hypersensitivity responses, this one is not. The antibodies that activate complement are IgM and IgG. IgE does not activate complement.

3. **The correct answer is B.** This child has leukocyte adhesion deficiency (LAD), which is a genetic deficiency of CD18. CD18 is an essential component of a number of integrins, and absence of these molecules causes the inability of WBCs to migrate into sites of inflammation. Thus in this patient, the blood contained abnormally high numbers of neutrophils, but they were unable to extravasate. CD18 is a component of LFA-1, CR3, and CR4.

   Absence of CCR4 (**choice A**) would cause difficulties in extravasation and migration of activated T cells and monocytes. This chemokine receptor is not found on neutrophils and therefore would have no effect on neutrophil migration.

   Absence of interleukin-1 (**choice C**) might cause difficulties in producing the acute and chronic inflammatory responses. This cytokine, frequently referred to as the endogenous pyrogen, produces fever, acute phase protein production, and many other results critical to inflammation. However, the actions of IL-1 are extremely redundant with those of IL-6 and tumor necrosis factor-α, so such a condition might have no clinically observable effects.

   Absence of interleukin-4 (**choice D**) would result in defects in the ability to mount a normal IgE antibody response. This cytokine also serves as the major stimulus for the development of TH2 cells from naive helper T cells, so its absence would be likely to have profound effects on all aspects of the secondary antibody response.

   Absence of tumor necrosis factor-α (**choice E**) might cause difficulties in producing the acute and chronic inflammatory responses. This cytokine has many functions that are redundant with those of IL-1 and IL-6, so such a condition might have no clinically observable effects.

4. **The correct answer is D.** This child has chronic granulomatous disease (CGD). The history indicates he has had recurrent infections with catalase-positive organisms and has a defect in generating oxygen radicals intracellularly in his phagocytic cells (the negative nitroblue tetrazolium test and neutrophil oxidative index). This genetic defect arises from a failure to produce one of the subunits of NADPH oxidase, which makes the individual incapable of producing intracellular oxygen radicals. Redundant intracellular killing mechanisms (myeloperoxidase and lysosomal contents) are still functional in these patients, but when they are infected with catalase-positive organisms, the substrate for myeloperoxidase (hydrogen peroxide) is destroyed, and the only remaining intracellular killing mechanism (lysosomes) is insufficient to protect from infection.

   C3 deficiency (**choice A**) would cause increased susceptibility to pyogenic infections because C3b is an important opsonin that enhances phagocytosis of extracellular organisms. All extracellular bacteria would be included in this list, not simply catalase-positive ones, as mentioned here. The NBT and NOI would not be negative in this case.

   Deficiency of CD18 (**choice B**) is the cause of leukocyte adhesion deficiency (LAD). Because CD18 is the common β chain of the β_2 integrins, its absence compromises leukocyte function antigen (LFA)-1, as well as complement receptors 3 and 4. Patients with LAD suffer recurrent infections with extracellular pathogens (not just catalase-positive ones) because of defective opsonization, mobilization, adhesion, and chemotaxis. The NBT and NOI would be positive.

   Deficiency of myeloperoxidase (**choice C**) results from a deficiency of an important granule enzyme in phagocytic cells. However, because there are so many redundant mechanisms of intracellular killing, these patients generally have mild symptoms or none at all.

   A phagocyte granule structural defect (**choice E**) is responsible for the Chediak-Higashi syndrome. These patients have chemotactic and degranulation defects, lack NK activity, and have partial albinism.

5. **The correct answer is C.** The only substance on the list that is chemotactic for neutrophils is IL-8. Other neutrophil chemotactic factors that might have been mentioned would include C5a, fibrinopeptides, leukotriene B_4, and formyl methionyl peptides.

   Complement component 3b (**choice A**) is not chemotactic for neutrophils. It acts as an opsonin, enhancing the phagocytosis of coated particles.

   Immunoglobulin G (**choice B**) is not chemotactic for neutrophils. It acts as an opsonin, enhancing the phagocytosis of coated particles.

   Myeloperoxidase (**choice D**) is not chemotactic for neutrophils. It is an enzyme in the lysosomes of phagocytic cells that generates toxic halide radicals intracellularly when exposed to its substrate, hydrogen peroxide.

   Tumor necrosis factor-α (**choice E**) is not chemotactic for neutrophils. It is produced by macrophages and is involved with the production of chronic inflammation and cytotoxicity.

The Processing and Presentation of Antigen

6

What the USMLE Requires You to Know

- How the grooves of class I and II MHC are loaded with peptides
- The three signals required for T-cell activation (TCR binding, costimulatory molecules, and cytokines)
- The clinical results of MHC class II deficiency (bare lymphocyte syndrome)
- How superantigens act
- The subclasses of T helper (TH) cells, their functions, and regulation

Although some small, easily digestible antigens are almost totally degraded and exocytosed by phagocytes, as we saw in the last chapter, the critical first step in the elicitation of the adaptive immune response to a first antigenic challenge is the processing of such antigen for the presentation to naive T lymphocytes. Professional antigen-presenting cells (dendritic cells, macrophages) load partially degraded peptides they have ingested into the groove of the class II MHC molecule, so that this can be presented to T cells with idiotypes complementary to that structure. This is accomplished by the **endosomal (exogenous) pathway** of MHC loading.

When MHC class II molecules are produced in the endoplasmic reticulum of an antigen-presenting cell (APC), in addition to the α and β chains, a third chain called the **invariant chain** is synthesized at the same time. This blocks the peptide-binding groove so **no** normal cellular peptides can accidentally be attracted there. As the molecule is completed, it is transported in a vesicle to the location of endocytic vesicles containing the ingested internalized peptides. As these vesicles fuse, the invariant chain is degraded, and peptides in the vacuole are loaded into the MHC II groove. The MHC class II–peptide complex is then transported to the cell surface where it will be accessible for interaction with any T lymphocyte with a complementary TCR.

In A Nutshell

MHC class II molecules are loaded with peptides by the endosomal (exogenous) pathway.

Note

The invariant chain prevents any normal cellular peptides from being bound.

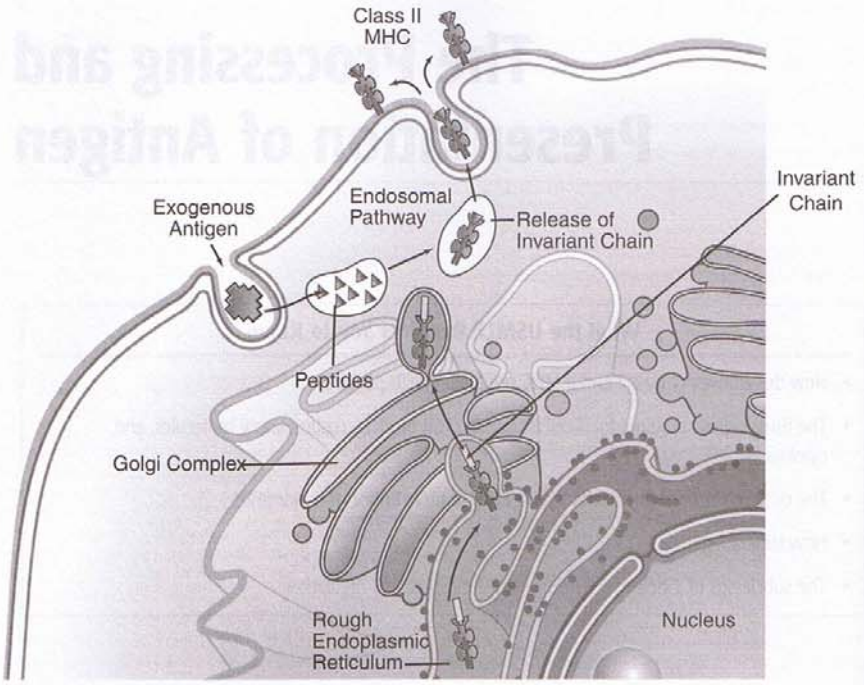


Figure II-6-1. The Exogenous Pathway of Binding Peptides to Class II MHC Molecules

In A Nutshell

- MHC I molecule is loaded with peptides via the endogenous pathway.
- CD8+ T cells recognize MHC I/peptide.

As with MHC class II molecules expressed on the surface of APCs and lymphocytes throughout the body, the MHC class I molecule presents small peptides in the groove at its N-terminal end, but the origin of these peptides is not from endocytic processing, but a different, **endogenous** pathway of production. Proteins synthesized in the cell cytosol are routinely degraded in proteasomes, and the peptides from these proteins are transported through a peptide transporter, known as the TAP complex, into the endoplasmic reticulum, where they have the opportunity to bind to freshly synthesized MHC class I proteins. These are then transported to the cell membrane where they may be presented to **CD8+ T lymphocytes** (*see* Chapter 8).

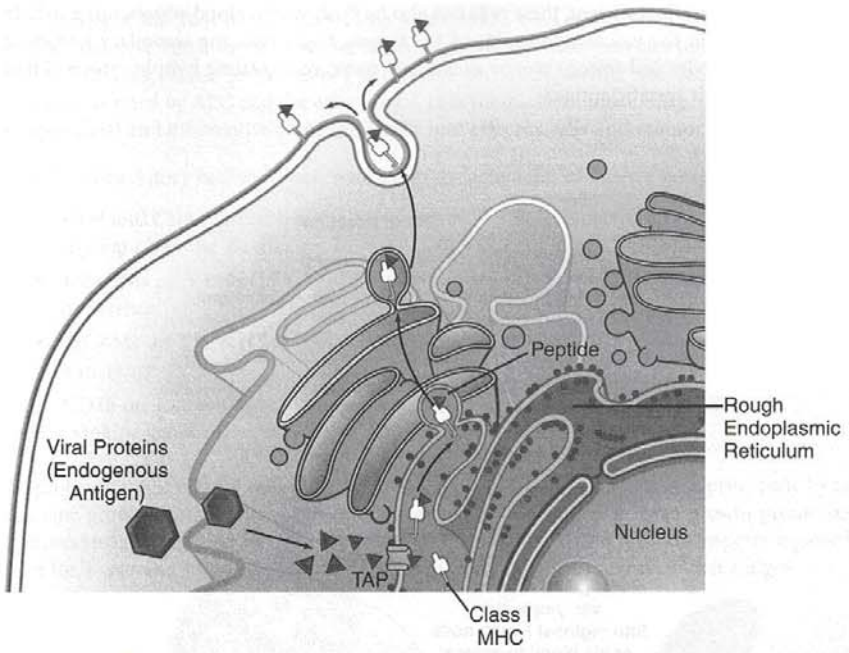


Figure II-6-2. The Endogenous Pathway of Binding Peptides to Class I MHC Molecules

Table II-6-1. Human MHC Summary

	MHC Class I	MHC Class II
Names	HLA-A, -B, and -C	HLA-DP, -DQ, -DR
Tissue distribution	All nucleated cells, platelets	B lymphocytes, monocytes, macrophages, dendritic cells, Langerhans cells, activated T cells, activated endothelial cells
Recognized by	Cytotoxic T cells (CD8+)	TH cells (CD4+)
Peptides bound	Endogenously synthesized	Exogenously processed
Function	Elimination of abnormal (infected) host cells by cytotoxic T cells	Presentation of foreign antigen to TH cells
Invariant chain	No	Yes
β_2 microglobulin	Yes	No

Within a few hours of the initiation of the acute inflammatory response by the breaching of the mucosa or epithelia, the professional APCs that have phagocytosed and processed the invading antigen begin to leave the area via lymphatic vessels. Dendritic cells, with their long, finger-like processes, are probably the most efficient of these cells and retract their membranous processes to round up and begin the journey to the closest lymph node. Thus, phagocytes with MHC class II molecules loaded with peptides digested from the invading antigen enter the lymph node through the afferent lymphatics and become trapped in the meshwork of the organ. If the

In A Nutshell

APCs with MHC class II/peptide molecules travel to the secondary lymphoid organs.

initial tissue damage is sufficient, these cells can also be flushed into blood vessels, ultimately to become trapped in the vascular sinusoids of the spleen. Regardless, the secondary lymphoid organs (lymph nodes and spleen) are the sites where naive, recirculating lymphocytes will first be exposed to their specific antigen.

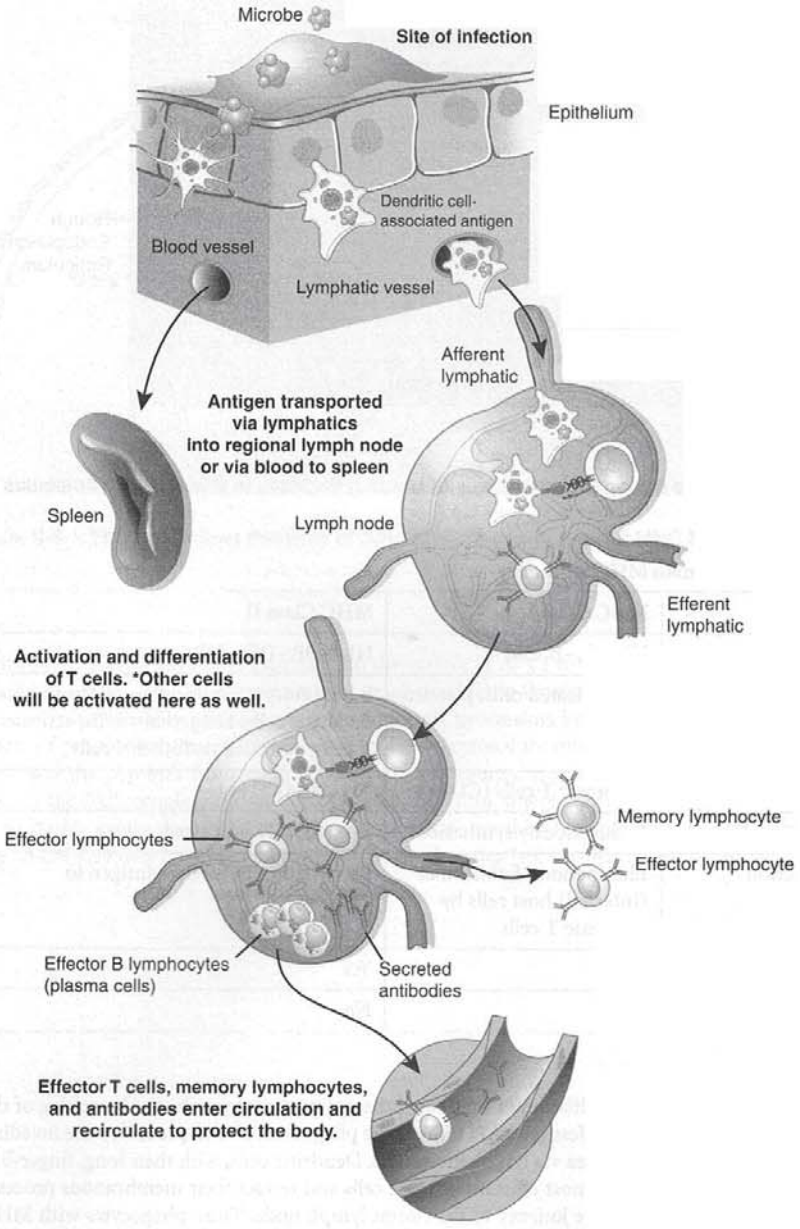

Microbe
Site of infection
Epithelium
Dendritic cell-associated antigen
Blood vessel
Lymphatic vessel
Afferent lymphatic
Antigen transported via lymphatics into regional lymph node or via blood to spleen
Spleen
Lymph node
Efferent lymphatic
Activation and differentiation of T cells. *Other cells will be activated here as well.
Memory lymphocyte
Effector lymphocytes
Effector lymphocyte
Effector B lymphocytes (plasma cells)
Secreted antibodies
Effector T cells, memory lymphocytes, and antibodies enter circulation and recirculate to protect the body.

Figure II-6-3. The Transportation of Antigen to the Secondary Lymphoid Organs

The binding of the TCR of the naive T cell to the MHC class II–peptide complex of the APC provides the first signal to the T cell to begin its activation. This provides the antigenic specificity of the response. Costimulatory molecules on APC provide the second signal, and cytokines secreted by APC and the activating T cells themselves induce the proliferation (**clonal expansion**) and differentiation of the T cells into **effector cells** and **memory cells**.

Several costimulatory molecules are involved in the activation of naive T lymphocytes:

- **CD4 and CD8** (coreceptors for MHC classes II and I, respectively) transduce activating signals to the T cells.
- **Integrins** on T cells (LFA-1) bind to IgCAMs on APCs (ICAM-1) to increase cell–cell adherence.
- **IgCAMs** on T cells (CD2) bind to integrins (LFA-3) on APCs to increase cell–cell adherence.
- **CD28** on T cells binds to B7 on APCs and triggers the transcription of several cytokine genes.

The proliferation of naive T cells in response to antigen recognition is mediated principally by an autocrine growth pathway, in which the responding T cell secretes its own growth-promoting cytokines and also expresses receptor molecules for these factors. IL-2 is the most important growth factor for T cells and stimulates the proliferation of clones of T cells specific to that antigen.

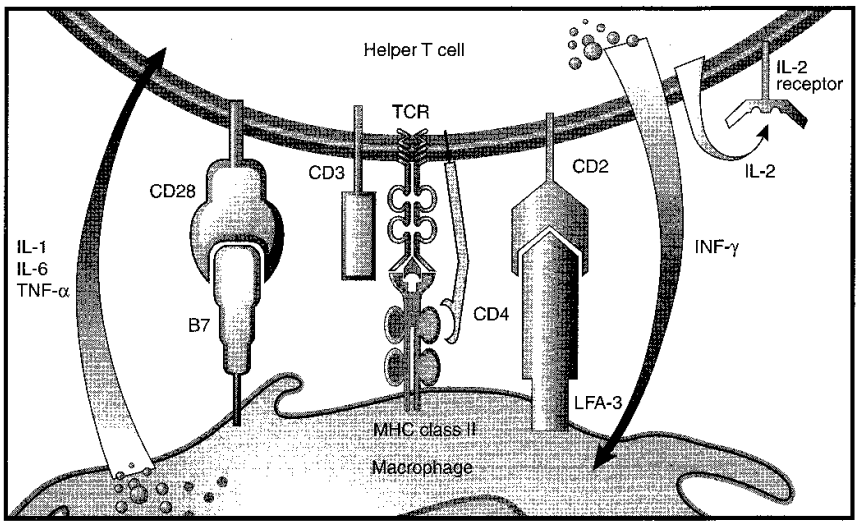


Figure II-6-4. Helper T Cell and Macrophage Adhesion

In A Nutshell

T-cell Activation–First signal = binding TCR to MHC II/peptide complex

In A Nutshell

Second Signal (Costimulatory Molecules)

- CD4 binds MHC II.
- CD8 binds MHC I.
- LFA-1 binds ICAM-1.
- CD2 binds LFA-3.
- CD28 binds B7.

In A Nutshell

Third Signal (Cytokines)

- IL-2
- IL-1
- IL-6
- TNF-α

Clinical Correlate

Superantigens are viral or bacterial proteins that cross-link the variable β domain of a T-cell receptor to an α chain of a class II MHC molecule. This cross-linkage provides an activating signal that induces T-cell activation and proliferation, in the absence of antigen-specific recognition of peptides in the MHC class II groove. Because superantigens bind outside of the antigen-binding cleft, they activate any clones of T cells expressing a particular variable β sequence and thus cause polyclonal activation of T cells, resulting in the overproduction of IFN-γ. This, in turn, activates macrophages, resulting in overexpression of proinflammatory cytokines (IL-1, IL-6 and TNF-α). Excess amounts of these cytokines induce systemic toxicity. Molecules produced during infectious processes and known to act as superantigens include staphylococcal enterotoxins, toxic-shock syndrome toxin-1 (TSST-1), and streptococcal pyrogenic exotoxins.

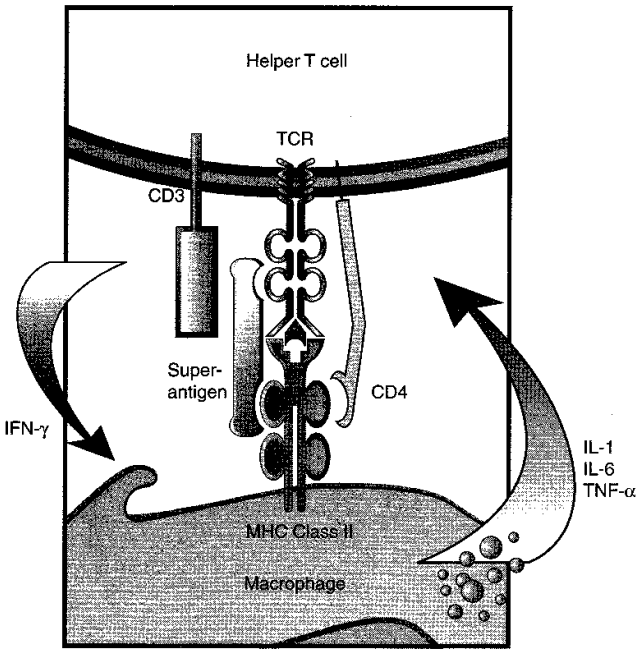


Figure II-6-5. Superantigen Activation of a Macrophage

In A Nutshell

TH cells control effector mechanisms of immunity (antibody, macrophage activation, CTL killing, and NK killing).

The activated CD4+ (helper) T lymphocytes, which have thus been generated in the lymph nodes and spleen following antigen administration, are now ready to serve as the orchestrators of virtually all the possible **effector mechanisms** that will arise to destroy the antigenic invaders. The effector mechanisms that are controlled totally or at least in part by TH cells include antibody synthesis, macrophage activation, cytotoxic T-cell killing, and NK cell killing. The "decision" as to which of these mechanisms should be encouraged is a function of the characteristics of the invading pathogen and is controlled by the differentiation of specialized classes of helper cells.

Clinical Correlate

Bare Lymphocyte Syndrome

Rare cases of MHC class II molecule deficiency (also known as **bare lymphocyte syndrome**) inherited in an autosomal-recessive fashion have been observed in humans. **Immune problems tend** to appear early in infancy and present as a mild form of severe combined **immunodeficiency (SCID;** *see* Chapter 11) with increased susceptibility to pyogenic and opportunistic **infections. However, these** defects can be distinguished from true SCID in that these patients will have **T cells that can respond** to nonspecific T-cell mitogens, such as phytohemagglutinin, and also to stimuli **with allogeneic cells** (cells from genetically nonidentical human beings). They do not develop graft-versus-host disease when given HLA-mismatched bone marrow transplants (*see* Chapter 14) because they do not express the MHC class II molecules against which such grafted cells can react. Patients with MHC class II deficiency are deficient in CD4+ cells due to failure of positive selection in the thymus, and they have moderate to severe hypogammaglobulinemia. This defect results from defects in the transcription factors required to coordinate their expression on the cell surface. Because MHC class I antigens are expressed normally, they do have CD8+ cells, although their function is diminished by the absence of TH1 cell cytokines.

There are two major classes of helper T (TH) cells, both of which arise from the same precursor, the naive TH lymphocyte, sometimes called the TH0 cell. The pattern of differentiation is determined by the stimuli present early in the immune response, at the site of antigen introduction. Differentiation of a TH0 cell into a TH1 cell seems to be stimulated by microbes that stimulate a strong initial innate immune response with the resultant production of IL-12 by macrophages or IFN-γ by NK cells. The differentiation of TH1 cells is stimulated by many intracellular bacteria, such as *Listeria* and mycobacteria, and by some parasites such as *Leishmania*—all of which infect macrophages. Differentiation of a TH0 cell into a TH2 cell seems to be encouraged in the absence of such innate immune stimuli, perhaps by default when persistent antigen remains in the system without significant macrophage or NK-cell stimulation. In this way, naive TH0 cells seem to produce IL-4 constitutively, and in the absence of IL-12 stimulation, these cells will upregulate their production of IL-4 to encourage differentiation into TH2 cells. The differentiation into the TH2 subset seems to be favored in infections with helminths and in response to allergens.

These classes of TH cells are distinguished almost entirely on the basis of the cytokines they produce. IFN-γ, TNF-β, and IL-2 are produced by TH1 cells, and IL-2, IL-4, IL-5, IL-6, and IL-10 are the cytokines produced by TH2 cells. These cytokines not only determine the stimulatory pathways that the cells will employ, but they also expand and develop the cells of the respective subset. For example, IFN-γ produced by TH1 cells promotes further TH1 development and inhibits the proliferation of TH2 cells. IL-4 and IL-10 produced by TH2 cells promote TH2 differentiation and inhibit the activation of TH1 cells. Thus, each subset amplifies itself and cross-regulates the other set so that immune responses become increasingly polarized over time, reaching extremes in cases where antigen exposure becomes chronic.

In A Nutshell

- TH1 cells arise from precursor when a strong innate immune response is stimulated.
- TH2 cells arise in the absence of such responses.

In A Nutshell

- TH1 cells produce IFN-γ, TNF-β, and IL-2.
- TH2 cells produce IL-2, IL-4, IL-5, IL-6, and IL-10.
- IFN-γ produced by TH1 inhibits TH2.
- IL-4 and IL-10 produced by TH2 inhibit TH1.

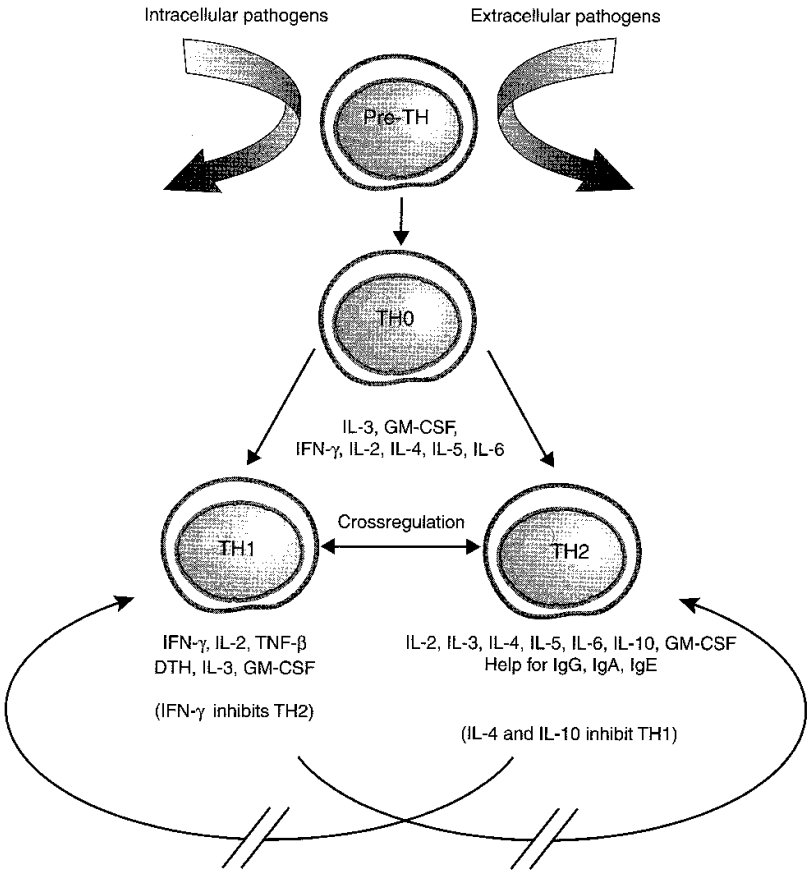


Figure II-6-6. Subsets of Helper T Cells

The effector mechanisms stimulated by the different classes of TH cells are specialized to optimally destroy different classes of invading antigens and are roughly (and somewhat artificially) divided into **cell-mediated** or **humoral** effector mechanisms.

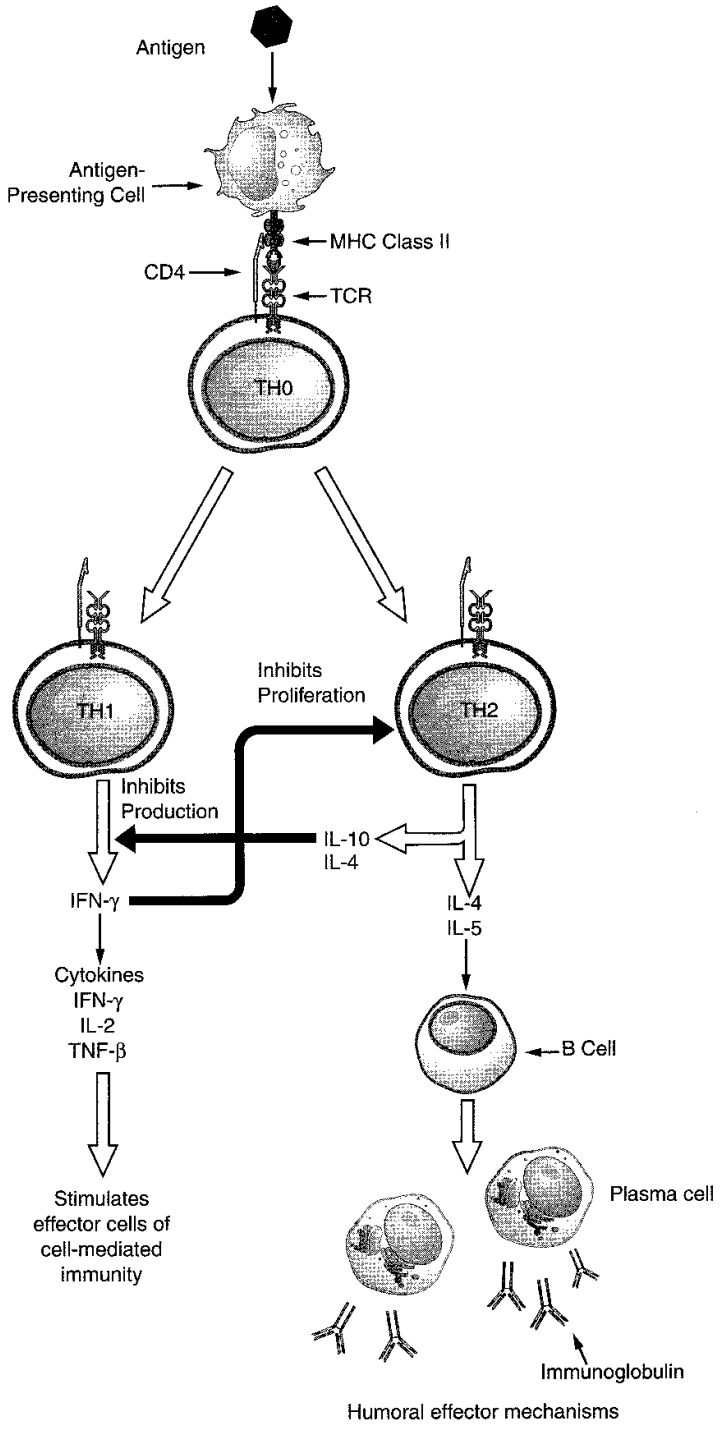


Figure II-6-7. Overview of T-Helper Cell Regulation of the Adaptive Immune Response

Clinical Correlate

Tuberculoid Versus Lepromatous Leprosy

The progression of disease with *Mycobacterium leprae* in humans is a well-documented example of the crucial balance between TH1 and TH2 subsets. Leprosy is not a single clinical entity, but presents as a spectrum of diseases, with tuberculoid and lepromatous forms being at the far poles. In **tuberculoid** leprosy, the patient has a **strong TH1** response, which eradicates the intracellular pathogens by granuloma formation. There is some damage to skin and peripheral nerves, but the disease progresses slowly, if at all, and the patient survives. In **lepromatous** leprosy, the **TH2 response is turned on**, and because of reciprocal inhibition, the cell-mediated response is depressed. These patients develop antibodies to the pathogen that are not protective, and the mycobacteria multiply inside macrophages, sometimes reaching levels of 10^{10} per gram of tissue. Hypergammaglobulinemia may occur, and these cases frequently progress to disseminated and disfiguring infections.

Chapter Summary

- Partially digested peptides are loaded into the groove of class II MHC molecules on antigen-presenting cells by the endosomal (exogenous) pathway.
- APCs migrate to the secondary lymphoid organs, where they present this processed antigen to recirculating naive lymphocytes.
- The binding of the TCR to the peptide/MHC class II complex provides the first signal in T-cell activation.
- Costimulatory molecule interactions (e.g., CD28 binds to B7, CD4 binds to MHC II, CD8 binds to MHC I, LFA-1 binds to ICAM-1, and CD2 binds to LFA-3) serve as the second signal in T-cell activation.
- Cytokines (IL-2, IL-1, IL-6, and TNF-α) serve as the final signal in T-cell activation.
- Superantigens are viral or bacterial proteins that cross-link the variable β domain of a T-cell receptor to an α chain of a class II MHC molecule and thereby cause polyclonal activation of T cells, overproduction of cytokines, and systemic toxicity.
- Activated TH cells act as the orchestrators of the effector mechanisms of the immune response (antibody synthesis, macrophage activation, cytotoxic T cell killing, and NK cell killing).
- Naive TH cells (TH0) differentiate into TH1 cells when IL-12 from macrophages or IFN-γ from NK cells is present. TH1 cells secrete IFN-γ, IL-2, and TNF-β.
- Naive TH0 cells differentiate into TH2 cells when there is extracellular attack. TH2 cells secrete IL-2, IL-4, IL-5, IL-6, and IL-10.
- The cytokines produced by TH subsets are cross-regulatory: IFN-γ produced by TH1 cells inhibits TH2 cells, and IL-4 and IL-10 produced by TH2 cells inhibit TH1 cells.

Review Questions

1. Human infections with *Mycobacterium leprae* express a spectrum of clinical presentations depending on the extent and expression of their immune response to the intracellular organism. On one end of the spectrum, patients with tuberculoid leprosy produce an effective cell-mediated immune response, which is successful at killing the intracellular organisms and, unfortunately, produces tissue damage. Patients with tuberculoid leprosy have granulomas that have elevated amounts of IL-2, IFN-γ, and TNF-β. The immune cell responsible for this pattern of cytokine production is the

   (A) Cytotoxic T lymphocyte
   (B) Epithelioid cell
   (C) Macrophage
   (D) TH1 cell
   (E) TH2 cell

2. There is evidence that the immunologic pathway that distinguishes the selection between the two polar forms of leprosy depends on the initial means of antigen presentation, as well as individual human differences in response. If early events of antigen recognition elicit production of IL-4, IL-5, IL-6, and IL-10, lepromatous leprosy is more likely to result, with the outcome of failure to mount a protective delayed-type hypersensitivity response. What differential characteristic of the lepromatous form is predicted based on the fact of overproduction of IL-4, IL-5, IL-6, and IL-10 in lepromatous lesions?

   (A) Autoimmunity
   (B) Granuloma formation
   (C) Hypergammaglobulinemia
   (D) Immediate hypersensitivity
   (E) Inflammation

3. A 10-month-old infant girl is admitted to the hospital with signs of *Pneumocystis carinii* pneumonia. Studies of her peripheral blood demonstrate age-normal counts of CD19+ cells, but CD3+ and CD4+ cell numbers are depressed. Immunoelectrophoresis of her serum reveals a moderate hypogammaglobulinemia. Her peripheral blood lymphocytes proliferate normally in response to phytohemagglutinin and MHC class I mismatched allogeneic cells. In a one-way mixed lymphocyte reaction using her cells as the stimulator cells, allogeneic T lymphocytes did not proliferate. Which of the following best describes the molecule most likely lacking from her lymphocytes?

   (A) It is designed to bind endogenously produced peptides
   (B) It is designed to bind exogenously processed peptides
   (C) It possesses β_2 microglobulin
   (D) It possesses two chains of unequal length
   (E) It should be present on all nucleated cells in the body

4. An elderly man with diabetes develops a blister on the heel of his foot, which becomes infected. Although nursing staff in the home where he is a resident clean and treat the wound with topical antibiotic ointment, he develops a fever and hypotension, and a desquamating rash spreads from the site of the original blister. How does the toxin responsible for his symptoms cause these signs?

   (A) It acts as an IL-1 homologue
   (B) It activates B lymphocytes polyclonally
   (C) It activates complement
   (D) It cross-links MHC class II molecules to TCRs polyclonally
   (E) It stimulates neutrophils

5. It has been learned in several experimental systems that proliferation and differentiation of T lymphocytes in response to tumor cells is low because tumor cells lack the necessary costimulatory molecules for lymphocyte activation. If melanoma cells from a patient were induced to express these costimulatory molecules by transfection, production of an effective antitumor response might occur. Which of the following molecules would be the best candidate for transfection of tumor cells to achieve this end?

   (A) B7
   (B) CD2
   (C) CD4
   (D) CD28
   (E) LFA-1

Answers and Explanations

1. **The correct answer is D.** IL-2, IFN-γ, and TNF-β are all elaborated by the TH1 cell. TNF-β can also be made by NK cells. In tuberculoid leprosy, the TH1 arm of the immune response is most active, resulting in a protective (but also damaging) cell-mediated response and a dampening of the antibody response. In lepromatous leprosy, the patient has an overabundance of TH2 responses, causing the production of a nonprotective antibody response.

   Cytotoxic T lymphocytes (**choice A**) are an effector cell in the cell-mediated immune response. They do not elaborate many cytokines but produce cytotoxic molecules, which cause the destruction of specific target cells.

   Epithelioid cells (**choice B**) are modified macrophages. They are extremely secretory and may produce IL-1, IL-6, TNF-α, IFN-γ, and GM-CSF. They are prominent in granulomas, and their cytokines would be elevated in a patient with tuberculoid leprosy, but that was not the question.

   Macrophages (**choice C**), once activated, may produce IL-1, IL-6, TNF-α, IFN-γ, and GM-CSF. They are prominent in granulomas, and their cytokines would be elevated in a patient with tuberculoid leprosy, but again, that was not the question.

   TH2 cells (**choice E**) would be elevated during lepromatous leprosy. The cytokines they secrete include GM-CSF, IL-2, IL-3, IL-4, IL-5, IL-6, and IL-10. These cells are stimulators of the humoral immune response.

2. **The correct answer is C.** In lepromatous leprosy, the activation of the TH2 arm of the immune response results in elicitation of those cytokines that stimulate production of antibody (IL-2, IL-4, IL-5, IL-6, and IL-10) and those that inhibit the development of the protective cell-mediated immune response (IL-4 and IL-10). Therefore, hypergammaglobulinemia is a frequent finding in lepromatous leprosy.

   Autoimmunity (**choice A**) may develop following infectious processes, but there is no evidence that stimulation of TH2 cells, by itself, causes autoimmune disease.

   Granuloma formation (**choice B**) would be decreased after exposure to these cytokines. Granulomas are an expression of the delayed-type hypersensitivity response, which is a function of TH1 cells. IL-10 and IL-4 would depress the TH1 response.

   Immediate hypersensitivity (**choice D**) requires sensitized mast cells and IgE antibodies. Although this result could occur in persons predisposed to atopic allergy, it is not the most likely result of stimulation with TH2 cytokines.

   Inflammation (**choice E**) is primarily mediated by substances released during tissue injury (leukotrienes, histamine, etc.) and the cytokines of activated macrophages (IL-1, IL-6, and TNF-α). It is not enhanced by TH2 cytokines.

3. **The correct answer is B.** This child has bare lymphocyte syndrome, a rare autosomal-recessive disease in which there is absence of MHC class II molecules on cells. Thus, her cells can recognize other cells as foreign and proliferate to T-cell mitogens, but they cannot be recognized by allogeneic lymphocytes because they do not express class II MHC antigens on their surface. The phrase which best describes the MHC class II molecule on this list is that it is designed to bind exogenously processed peptides. Other descriptions that could apply would be that it has two chains of similar length, is produced with an invariant chain, and is designed to present foreign peptides to TH cells.

   It is designed to bind endogenously produced peptides (**choice A**) is a description that fits the class I MHC molecule. If this were a case of class I MHC deficiency, she would not have made a normal proliferative response to mismatched allogeneic cells.

   It possesses β_2 microglobulin (**choice C**) is a description that fits the class I MHC molecule.

It possesses two chains of unequal length (**choice D**) is a description of the class I MHC molecule. It has an α chain with three domains, and a smaller chain, β_2 microglobulin, becomes associated with the α chain.

It should be present on all nucleated cells in the body (**choice E**) describes the class I MHC molecule. Class II MHC will be found on all antigen-presenting cells in the body.

4. **The correct answer is D.** This patient is showing signs of toxic shock syndrome, caused by infection of the blister with *Staphylococcus aureus* and the resultant elaboration of the exotoxin TSST-1. This toxin acts as a superantigen, cross-linking the variable β region of the TCR to the α chain of the class II MHC molecule. This binds TH cells and APC together without the specificity of antigen recognition, and so clonal proliferation of T cells and production of IFN-γ leads to activation of macrophages. As a result, the macrophages overproduce the cytokines IL-1, IL-6, and TNF-α, which are toxic at high levels.

   It acts as an IL-1 homologue (**choice A**) is not true. IL-1 is produced by macrophages as a result of T-cell activation, but TSST-1 does not itself act as an IL-1 homologue.

   It activates B lymphocytes polyclonally (**choice B**) is not true. TSST-1 acts on TH cells to stimulate macrophage cytokines. It does not have a direct effect on B-cell proliferation.

   It activates complement (**choice C**) is not correct. TSST-1 does not have an effect on complement.

   It stimulates neutrophils (**choice E**) is not correct. Although neutrophils are stimulated during *Staphylococcus aureus* infection and produce IL-1, which causes fever, the mechanism of action of TSST-1 and other superantigens is not through neutrophil activation.

5. **The correct answer is A.** The B7 molecule on antigen-presenting cells binds to the CD28 molecule on T lymphocytes and serves as a costimulatory signal for their activation. If the tumor cells could be induced to express this costimulatory molecule, they would provide the important activating signal to the T cells.

   CD2 (**choice B**) is the molecule on T lymphocytes that binds to LFA-3 on antigen-presenting cells. If the tumor cell were induced to express CD2, it would bind to the complementary structure on macrophages and not activate the T cells.

   CD4 (**choice C**) is the molecule on T lymphocytes that stabilizes the interaction of MHC class II and the TCR. If the tumor cell were induced to express CD4, it would not increase the tumor-specific response.

   CD28 (**choice D**) is the molecule on T cells that binds to B7. If the tumor cell were induced to express CD28, it would bind to the complementary structure on macrophages and not activate the T cells.

   LFA-1 (**choice E**) is the molecule on T cells that binds ICAM-1 on the antigen-presenting cells. If the tumor cells were induced to express LFA-1, it would bind to the complementary structure on macrophages and not activate the T cells.

The Generation of Humoral Effector Mechanisms

7

What the USMLE Requires You to Know

- The humoral responses to thymus-independent and -dependent antigens
- The cell surface molecules that contribute to production of T- and B-cell conjugates
- The basic structures and effector functions of the five antibody isotypes
- The meaning of valence, affinity, and avidity
- The role of somatic hypermutation in affinity maturation
- How the alternative and classical complement cascades are initiated
- The biologic functions of complement components

Humoral immunity is mediated by antibodies synthesized by B lymphocytes and secreted by their fully differentiated end cell, the **plasma cell.** This arm of the immune response is directed toward the defense against **extracellular** microbes or toxins and may culminate in the extracellular degradation of such materials or the enhancement of their destruction via phagocytosis.

As mature naive B lymphocytes leave the bone marrow following successful rearrangement of their membrane immunoglobulin receptor genes, they recirculate throughout the body, attracted to **follicular areas** of the lymph nodes and spleen. If antigen entering these secondary lymphoid organs binds to and cross-links the idiotypes of these membrane receptors, this provides the first signal for the activation of the B lymphocyte.

Most antigens introduced into the body fall into the category of **thymus-dependent antigens.** Response to such molecules requires the direct contact of B cells with TH cells and their **cytokines.** After the cross-linking of receptors on the B-cell surface with antigen, the material is endocytosed and processed via the endocytic (exogenous) pathway to generate MHC class II/peptide conjugates, which are then inserted in the membrane, just as we have seen during phagocytosis and processing in the professional phagocyte lineages. Simultaneously, expression of costimulatory molecules such as B7 is upregulated on the B lymphocyte, making them effective presenters of antigen to TH cells in the area. Once a TH cell recognizes a processed antigenic peptide displayed with MHC class II molecules on the membrane of the B cell, the two cells form a **conjugate,** and the TH cell is activated and induced to become a TH2 cell. TH2 cells in conjugates rearrange their Golgi apparatus toward the junction with the B cell leading to the directional release of cytokines toward the B cell. In addition, expression of a molecule known as CD40L on the surface of the TH2 cell is upregulated, and this molecule interacts with CD40 on the B cell to provide the second signal for B-cell activation. The B cells are encouraged to proliferate after this interaction. The final signal delivered by the TH2 cell is the release of cytokines, which will induce the differentiation of B cells into fully differentiated, antibody-secreting cells and memory cells (*see* Chapter 9) and induce **class switching.**

In A Nutshell

- Humoral immunity = antibodies
- Defends against extracellular agents
- B lymphocytes are attracted to follicular areas of secondary lymphoid organs.
- Most pathogens are thymus-dependent antigens.

In A Nutshell

B-cell contact with TH cells requires:

- MHC II/peptide presentation
- Costimulatory molecules (B7)
- CD40/CD40L binding

In A Nutshell

TH2 cytokines induce B-cell:

- Differentiation
- Memory
- Class switching

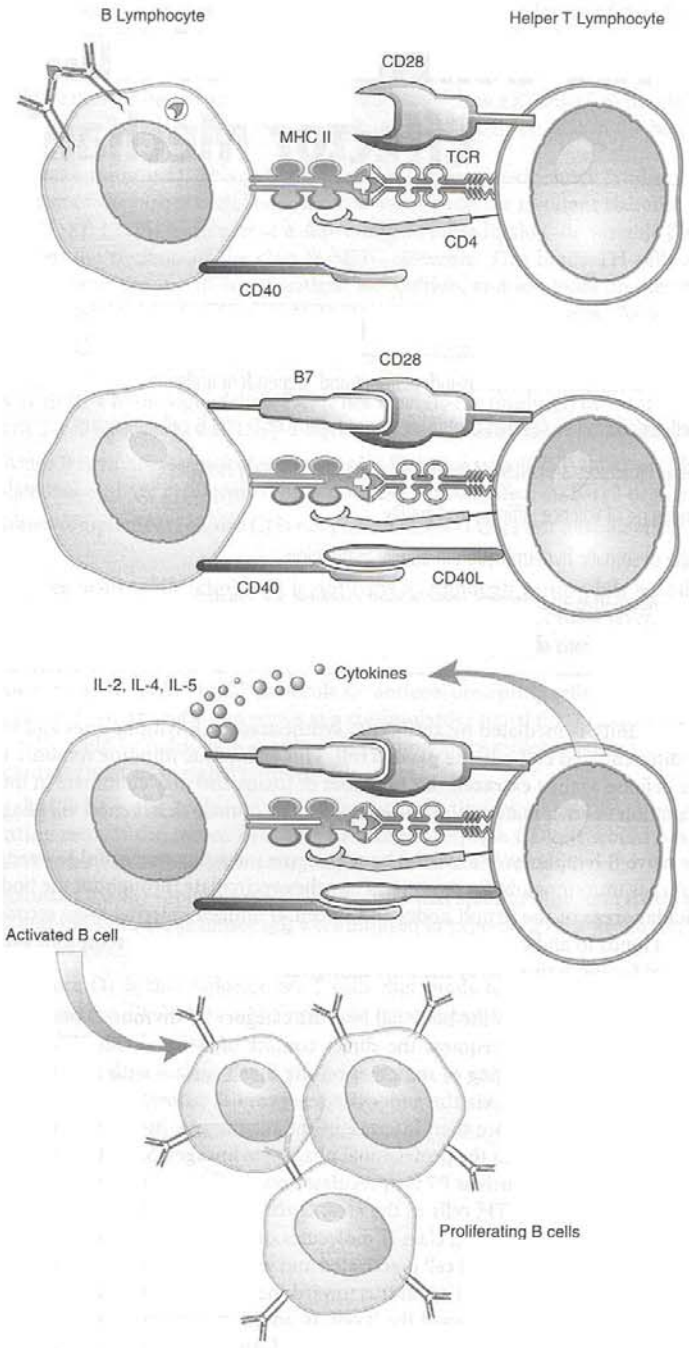


Figure II-7-1. The Formation of T- and B-Cell Conjugates

T cells recognize peptides bound to MHC molecules. Therefore, antigens that possess no peptide structure cannot be recognized by T cells. These antigens are called **thymus-independent antigens** and include lipopolysaccharide from the cell envelope of gram-negative bacteria and polysaccharide capsular antigens. These antigens may directly stimulate B cells to cause proliferation and secretion of antibody, or they may act as B-cell **mitogens**, directly activating these cells regardless of their antigenic specificity. The response to thymus-independent antigens is generally **weaker** than the response to other classes of antigens, resulting in the **secretion of IgM antibodies only** and the **absence of immunologic memory.**

Although all of the antibody molecules secreted by a clone of B lymphocytes will have identical idiotypes (*see* Chapter 2), the B cell is induced to make new classes, or **isotypes**, of immunoglobulin in response to cytokine-directed instruction from the TH2 cell. The progression of new antibody isotypes produced by B cells is defined by the sequence of constant domain coding in the B-lymphocyte DNA, and each isotype of immunoglobulin is designed with a different effector function in mind. Just as the three-dimensional structure of the **idiotype defines antigen specificity**, the sequence of amino acids in the constant domains of the immunoglobulin molecule (**isotype**) **dictates the effector functions** that will be expressed.

The biologic function of segments of the antibody molecule was first elucidated by digestion of these molecules with proteolytic enzymes. If an antibody molecule is digested with **papain,** cleavage occurs above the disulfide bonds that hold the heavy chains together. This generates three separate fragments, two of which are called Fab (fragment antigen binding), and one is called Fc (fragment crystallizable). Cleavage of the antibody molecule with **pepsin** generates one large fragment called $F(ab')_2$ and a digested Fc fragment. The **bridging of antigens** by antibody molecules is required for **agglutination of particulate antigens** or the **precipitation of soluble antigens.**

In A Nutshell

Thymus-independent antigens:

- contain no peptides.
- stimulate only IgM.
- create no memory.

Note

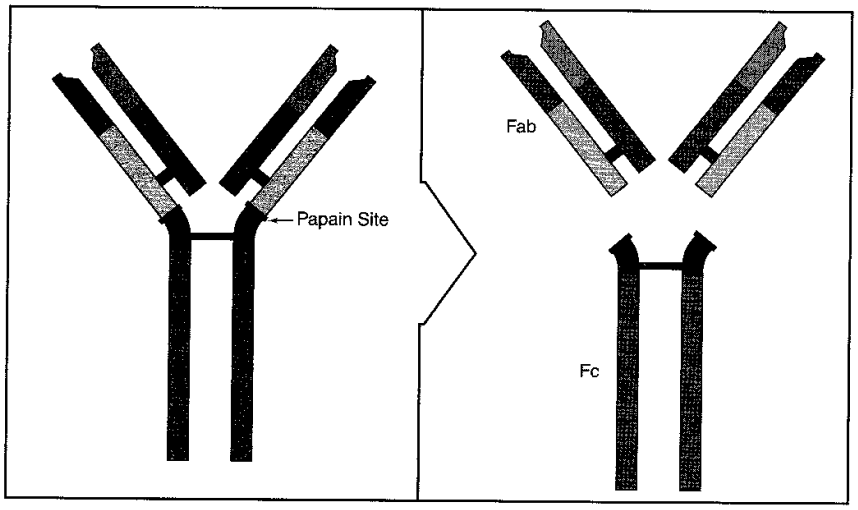
Mitogens activate many clones of B cells and are used clinically to assess lymphocyte function.

In A Nutshell

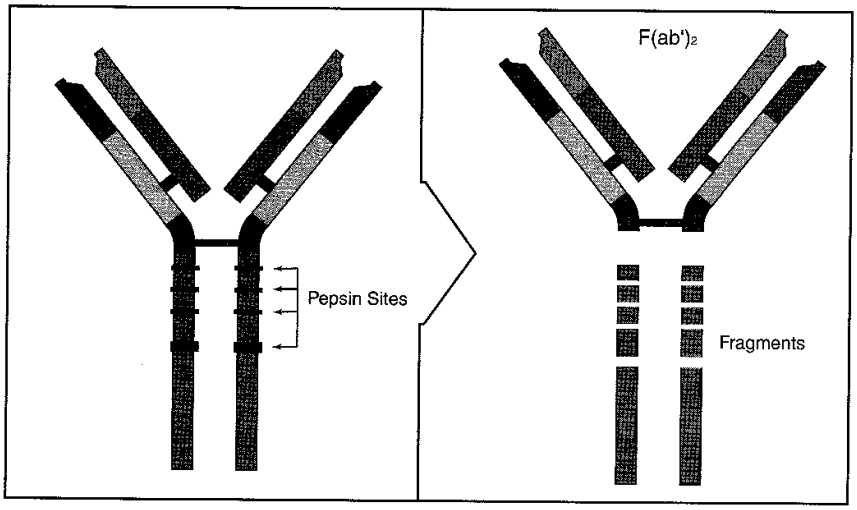
- Isotype switching is directed by TH2 cells.
- Isotypes dictate effector function of the antibody molecule.

In A Nutshell

- Papain generates 2 Fab + 1 Fc.
  - Fab (monovalent)—capable of binding
- Pepsin generates 1 $F(ab')_2$.
  - $F(ab')_2$ (divalent)—capable of binding and bridging



Proteolytic Cleavage with Papain



Proteolytic Cleavage with Pepsin

Figure II-7-2. Proteolytic Cleavage of Immunoglobulin by Papain/Pepsin

In A Nutshell

- IgM is the first isotype produced.
- Plasma IgM exists as a pentamer.
- J chain joins the monomer units.

The first isotype of immunoglobulin that can be produced by a B cell with or without T-cell help is IgM. This is because coding for the constant domains of the heavy chain of IgM (μ chains) are the first sequences downstream from the coding for the idiotype of the molecule. The IgM molecule on the surface of the B cell is a monomer, but the secreted form of this molecule is a **pentamer**, held together in an extremely compact form by a **J chain** synthesized by the cell.

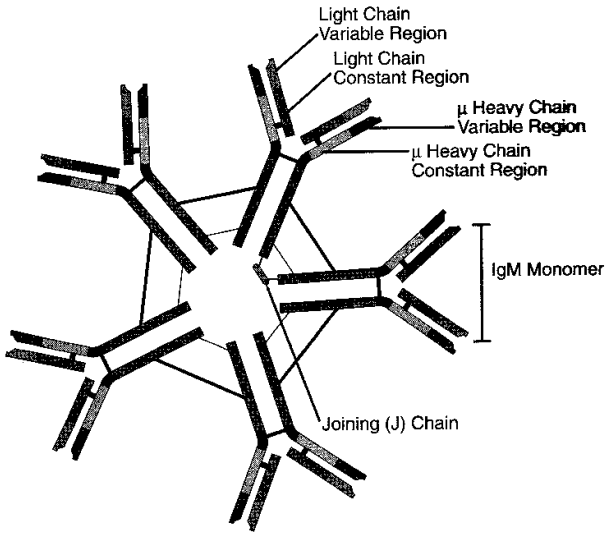


Figure II-7-3. The IgM Pentamer

The design of the IgM pentamer maximizes the effector functions critical to the body early during antigenic challenge. Because of its multimeric structure (5 of the Y-shaped monomers joined into one unit), plasma IgM has five times the capacity for binding antigenic epitopes as any monomeric immunoglobulin unit. The valence of the molecule is therefore 10: In other words, 10 identical epitopes can be simultaneously bound, as compared with 2 for the monomeric structure. This makes IgM the most effective immunoglobulin isotype at "sponging" the free antigen out of the tissues and proves critical, as the humoral response evolves, in trapping antigen so that it can be presented to the lymphocytes, which will ultimately refine the choice of effector mechanism. Although the binding strength (affinity) of the idiotype for the epitope may not be strong early in the immune response, the IgM molecule possesses the highest avidity (number of combining sites available to bind epitopes) of any immunoglobulin molecule produced in the body.

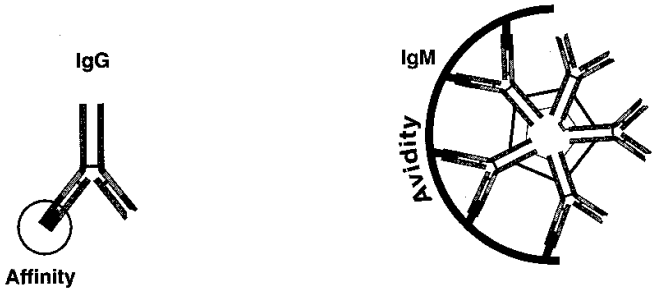


Figure II-7-4. Affinity and Avidity

In A Nutshell

IgM

- Plasma IgM valence = 10
- Functions in trapping free antigen
- Affinity (binding strength) may be low
- Avidity (multipoint binding) highest of all isotypes

In A Nutshell

- IgM is most effective isotype at activating complement.
- It is not an opsonin.
- It does not mediate ADCC.

Note

Isotype switching is induced by TH2 cells.

The multimeric structure of IgM also makes it the most effective antibody at activating complement, a set of serum proteases important in mediating inflammation and antigen removal. Serum IgM is incapable of binding to cellular Fc receptors and thus cannot act as an opsonin (*see* Chapter 5) or a mediator of antibody-dependent cell-mediated cytotoxicity (ADCC) (*see* Chapter 8).

As the B lymphocyte receives cytokine signals from the activated TH2 cells in the secondary lymphoid organs, it is induced to undergo **isotype switching**, changing the heavy-chain constant domains to classes of antibodies with new and different effector functions. It does this by rearranging the DNA encoding the constant region of the heavy chain by activating switch regions that cause the intervening DNA to be looped out, excised, and degraded. The idiotype is then joined to a new constant region domain coding, and an antibody molecule with identical antigenic specificity but a new effector function is produced. This isotype switch is **one-way**: Because the excised DNA is degraded, a cell that has begun to produce an isotype downstream from IgM coding can never produce IgM again.

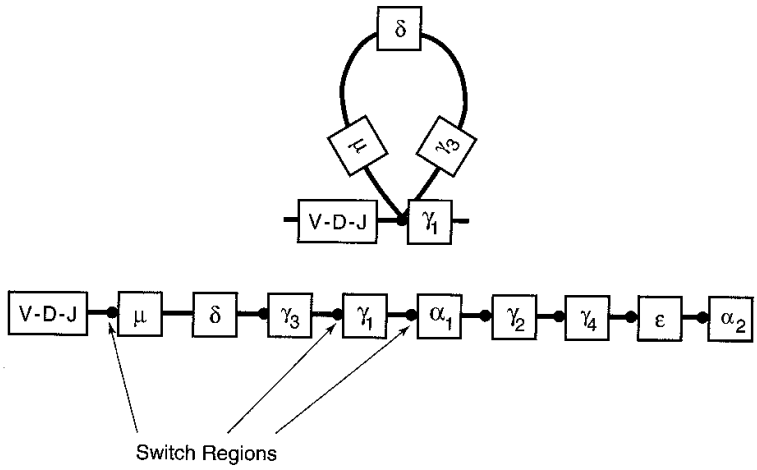


Figure II-7-5. Immunoglobulin Heavy Chain Switching

Note

IgM is the main immunoglobulin of the primary immune response.

This is why IgM is the principal immunoglobulin of the **primary immune response** when antigen is first encountered, and it is replaced in later responses by antibodies of different isotypes. Although IgM antibodies are occasionally produced at low levels during secondary and later immunologic responses, they are always produced by cells seeing that antigen for the first time; namely, naive cells newly emerging from the bone marrow (*see* Chapter 9).

Clinical Correlate

- IgM is used as a measure of a primary response (acute infection).
- Convalescent serum will have mostly IgG with subthreshold levels of IgM.

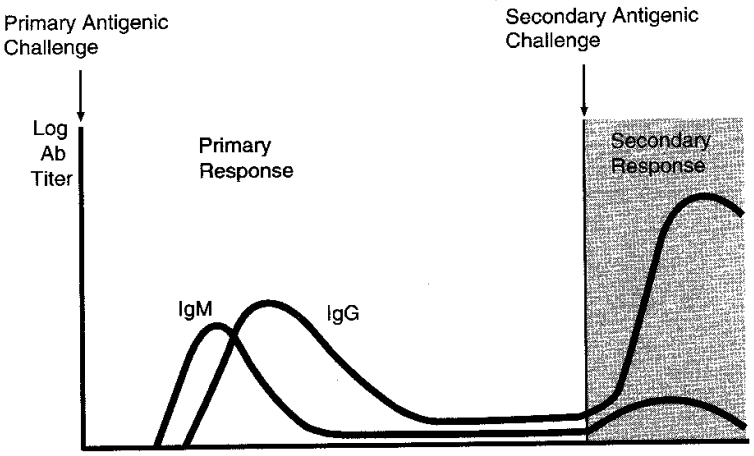


Figure II-7-6. Primary and Secondary Antibody Responses

During the activation of B lymphocytes by TH2 cells, intense proliferation of the B cells results in the formation of **germinal centers** in the follicles of the lymph nodes and spleen. These are clones of proliferating, antigen-specific cells. During the intense proliferative response of the B cell, random mutations in the coding of the variable domain region may occur. This is called **somatic hypermutation** and creates single point mutations in the antibody idiotype. If these slightly altered idiotypes have increased affinity for the antigen, then the cell expressing them will be at a selective advantage in competing to bind antigen. Because binding antigen serves as the first signal for proliferation, over time, clones of cells with higher receptor affinity will begin to predominate in the germinal center. This **clonal selection** results in the predominance of clones capable of producing antibodies with increasing affinity for the antigen, a process known as **affinity maturation.** This means that although isotype switching will necessarily **decrease the avidity** of the preponderance of antibody molecules as the immune response evolves, this will be substituted by an **increase in antibody affinity** over time.

The choice of activation of particular switch regions in the B-cell DNA sequence is apparently dictated by the release of specific cytokines by activated TH2 cells.

In A Nutshell

- Germinal centers are clones of proliferating antigen-specific B cells.
- Somatic hypermutation may cause minor idiotype changes.
- Clonal selection by competition for antigen causes affinity maturation.

In A Nutshell

TH2 cytokines dictate switch region activation.

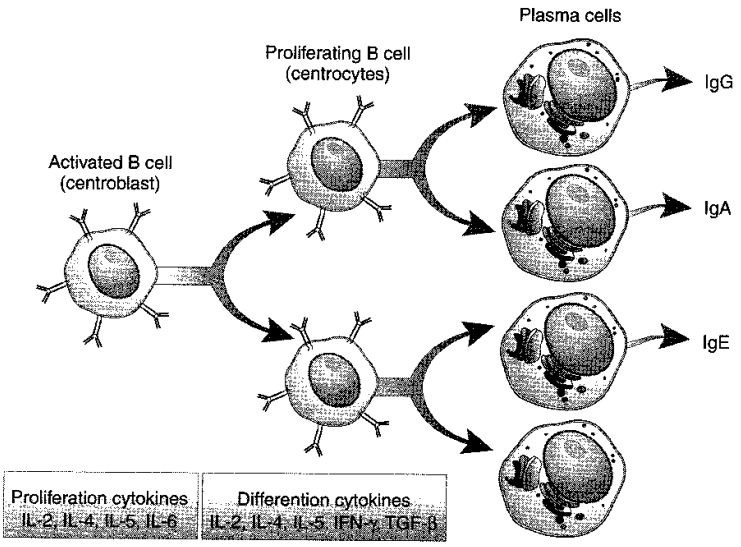


Figure II-7-7. TH-Cell Direction of B-Lymphocyte Proliferation and Differentiation

Clinical Correlate

X-Linked Hyper-IgM Syndrome is characterized by a deficiency of IgG, IgA, and IgE and elevated levels of IgM. IgM levels can reach 10 mg/mL (normal is 1.5 mg/mL). It is most commonly inherited as an X-linked recessive disorder, but some forms seem to be acquired and can be seen in both sexes. The peripheral blood of infected individuals has high numbers of IgM-secreting plasma cells, as well as autoantibodies to neutrophils, platelets, and red blood cells. These patients fail to make germinal centers during a humoral immune response. Children with this condition suffer recurrent respiratory infections, especially those caused by *Pneumocystis carinii*.

The defect in this syndrome is in the gene encoding the CD40 ligand, which maps to the X chromosome. Therefore, TH cells from these patients will fail to express functional CD40L on their membrane and will thereby fail to give the costimulatory signal necessary for the B-cell response to T-dependent antigens, so only IgM antibodies are produced. The B-cell response to T-independent antigens is unaffected.

In A Nutshell

- IgG is the major antibody produced after IgM.
- IgG exists in four subisotypes.
- IgG activates complement, opsonizes, and mediates ADCC.
- IgG is actively transported across the placenta.

The preponderant isotype of immunoglobulin that begins to be produced after IgM during the primary immune response is IgG. IgG is a monomeric molecule with a γ **heavy chain** and a new set of effector functions. IgG exists in four different **subisotypes** (subclasses) in humans—IgG1, -2, -3 and -4, each of which exhibits slightly different capacity in effector functions. But in general, IgG activates complement, acts as an opsonin, and mediates ADCC. It is also actively transported across the placenta by receptor-mediated transport and thus plays a crucial role in protection of the fetus during gestation.

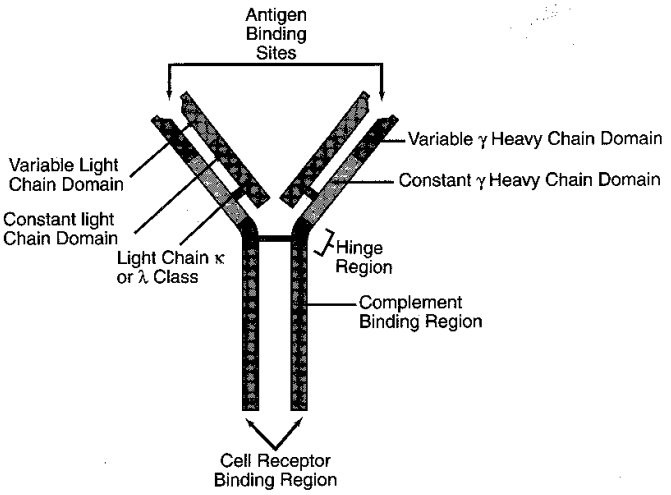

Antigen Binding Sites
Variable γ Heavy Chain Domain
Constant γ Heavy Chain Domain
Variable Light Chain Domain
Constant light Chain Domain
Light Chain κ or λ Class
Hinge Region
Complement Binding Region
Cell Receptor Binding Region

Figure II-7-8. The Basic Structure of IgG

Another isotype of antibody that can be produced following class switching is IgA, although this isotype is much more commonly produced in the submucosa than in the lymph nodes and spleen (*see* Chapter 9). IgA generally exists as a **dimer**, held together by a J chain similar to that produced with IgM, and serves as a major protective **defense of the mucosal surfaces of the body**. Its sole function appears to be the inhibition of binding of toxins or adhesive microbial components to the mucosa of the digestive, respiratory, and urogenital systems, and it does not activate complement or act as an opsonin.

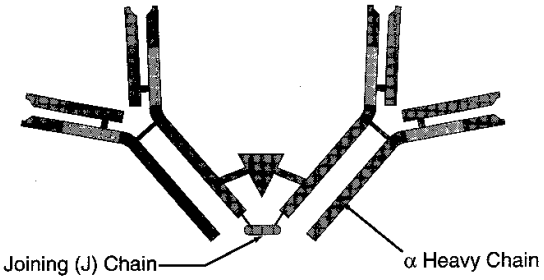


Figure II-7-9. The IgA Dimer

IgE is the so-called homocytotropic antibody because it binds to directly to Fcε receptors present on mast cells and basophils (without binding antigen) and is involved in elicitation of protective immune responses against helminth parasites and many allergic responses (*see* Chapter 13). It does not activate complement or act as an opsonin. Its heavy chain is called an ε chain.

In A Nutshell

- Most IgA is produced in the submucosa.
- IgA is a dimer with a J chain.
- IgA inhibits binding of adhesive substances to mucosal surfaces.
- IgA is an important component in breast milk.

In A Nutshell

- IgE is bound to mast cells and basophils.
- IgE mediates immediate type I allergic reactions.
- IgE protects against parasites.

Clinical Correlate

Immunodeficiencies Involving B Lymphocytes

Patients with B-cell deficiencies usually present with recurrent pyogenic infections with extracellular pathogens. The absence of immunoglobulins for opsonization and complement activation is a major problem (see Chapter 11). The T-cell immune system is intact, and T-cell activities against intracellular pathogens, delayed-type hypersensitivity, and tumor rejection are normal (see Chapter 8).

Table II-7-1. Summary of the Biologic Functions of the Antibody Isotypes

	IgM	IgG	IgA	IgD	IgE
Heavy chain	μ	γ	α	δ	ε
Adult serum levels (in mg/dL)	40–345	650–1,500	75–390	Trace	Trace
Functions					
Complement activation, classic pathway	+	+	–	–	–
Opsonization	–	+	–	–	–
Antibody-dependent cell-mediated cytotoxicity (ADCC)	–	+	–	–	–
Placental transport	–	+	–	–	–
Naive B-cell antigen receptor	+	–	–	+	–
Memory B-cell antigen receptor (one only)	–	+	+	–	+
Trigger mast cell granule release	–	–	–	-	+

In A Nutshell

The Complement System

- Has two pathways of activation
- Enhances inflammation
- Enhances phagocytosis
- Causes lysis

Complement

The **complement system** is a set of interacting proteins released into the blood after production in the liver. The components act together as zymogens, activating one another in cascade fashion after initiation from a variety of stimuli. Two different pathways of activation occur in the body and culminate similarly in the production of important split products that mediate **inflammation**, enhance phagocytosis by **opsonization**, and cause **lysis** of particles by membrane pore formation.

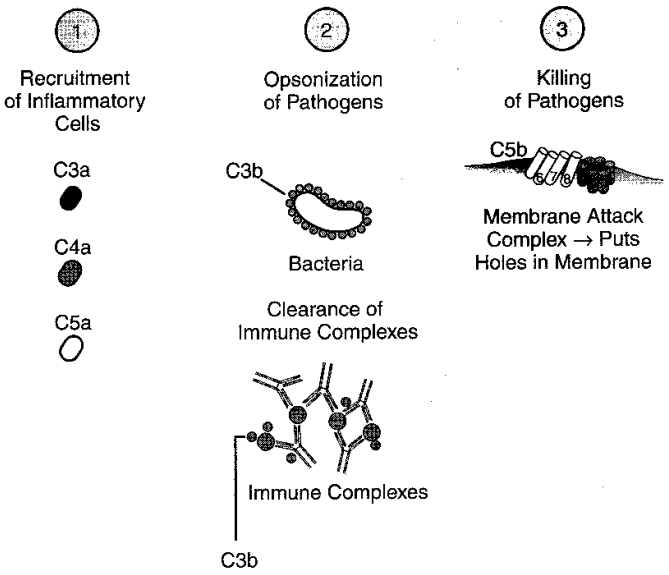


Figure II-7-10. Three Functions of the Complement System

The **alternative pathway** of complement activation is probably the more primitive of the two pathways because it is initiated by simple attraction of the early factors to the surfaces of microbes. Bacterial polysaccharides and the lipopolysaccharide of the cell envelope of gram-negative bacteria both serve as potent, initiating stimuli.

In A Nutshell

The alternative pathway is initiated by surfaces of pathogens.

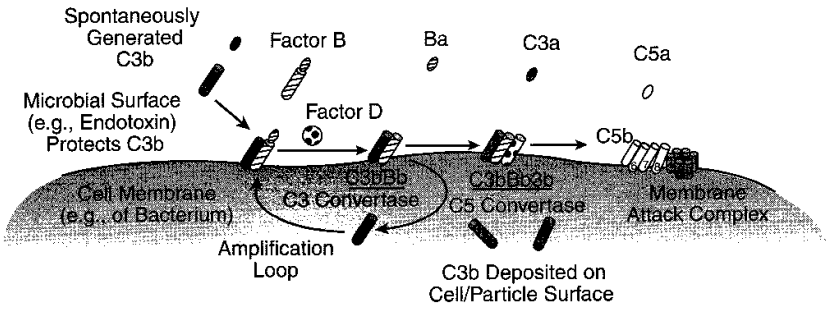


Figure II-7-11. The Alternative Complement Pathway

The **classical pathway** is activated by antigen-antibody complexes and is probably the more phylogenetically advanced system of activation. Both IgG and IgM can activate the system by this pathway, although IgM is the most efficient.

Note

The classical pathway is activated by Ag/Ab complexes.

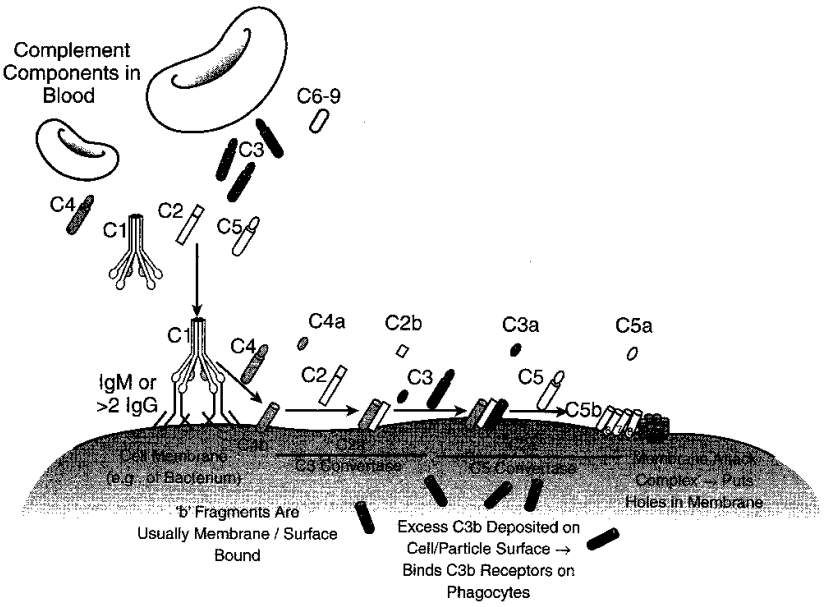


Figure II-7-12. The Classical Complement Pathway

Although the complement cascade is considered a constituent of the innate immune response, its overlapping stimulation of effector functions of cells of the adaptive immune response, as well as its role in enhancement of inflammation, make it a critical effector system for removal of extracellular invaders and concentration of antigens into the secondary lymphoid organs, where the adaptive immune responses are elicited.

In A Nutshell

Physiologic controls on complement activation act at level of C1, C3, and C5.

When uncontrolled activation of complement occurs in certain disease states (*see* Chapters 11 and 13), damage to host tissues can ensue. Physiologic controls on complement activation occur at the level of C1 (classical pathway) and C3 and C5 (both pathways).

Clinical Correlate

Complement Deficiencies

Genetic deficiencies have been described for each of the components of complement and their regulatory proteins (*see* Chapter 11). These deficiencies highlight the critical role of the early components of complement in generating C3b and the essential role of C3b for clearance of immune complexes from the body. Furthermore, even though gram-positive bacteria may be resistant to the membrane attack complex of complement, the early components of the cascade mediate localized inflammation and opsonize the bacteria. In a great number of cases then, it is the complement cascade that converts immunoglobulins into powerful effectors of bacterial destruction.

When deficiencies of complement regulatory components occur, then the uncontrolled activation of the complement cascade can have dangerous results in the body's own innocent bystanders. In hereditary angioedema, uncontrolled complement activation at the mucosal surfaces causes edema and pain. In paroxysmal nocturnal hemoglobinuria, the absence of regulatory proteins causes paroxysms of hemolysis of RBCs and the resultant hemoglobinuria.

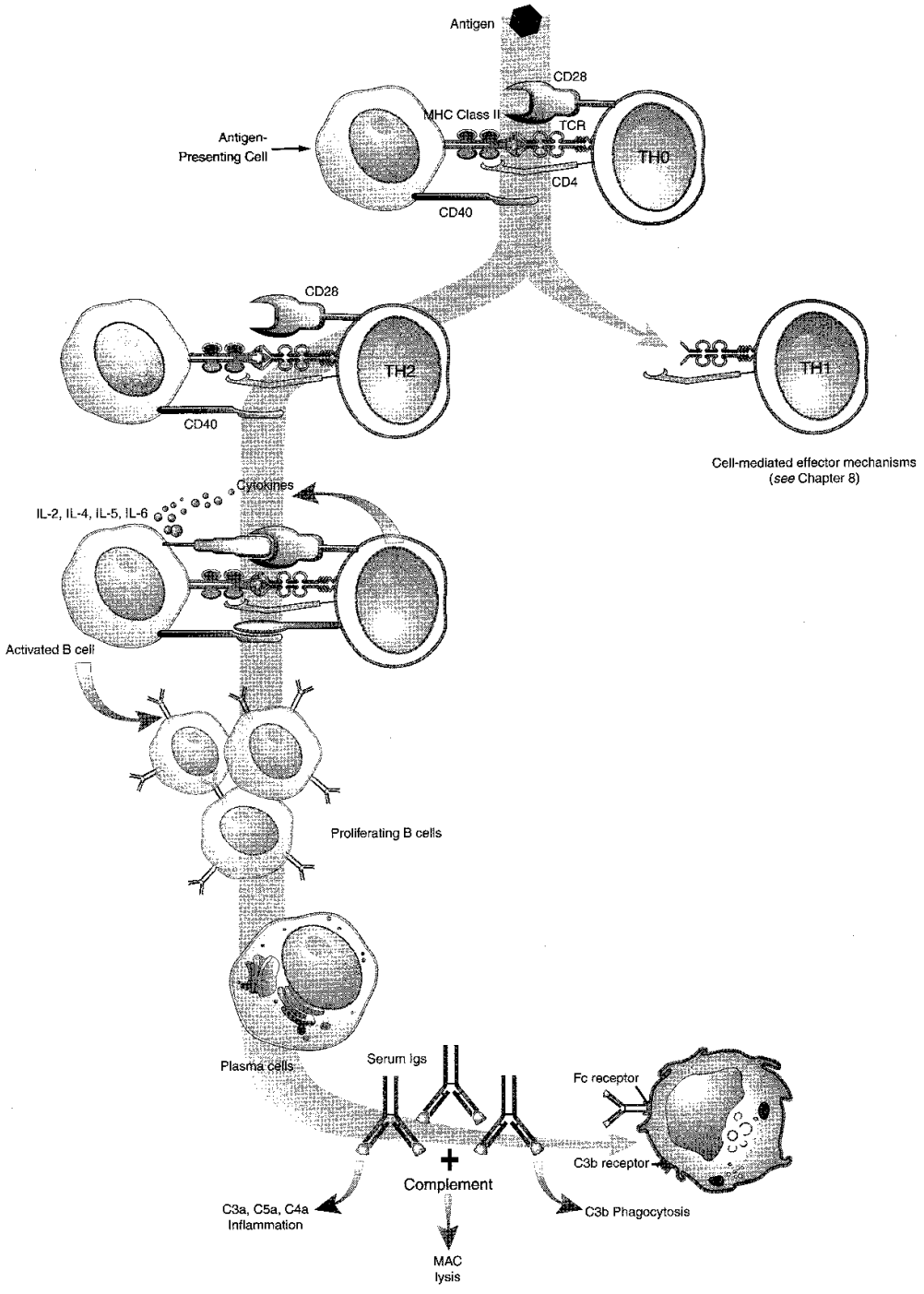


Figure II-7-13. Overview of the Generation of Humoral Effector Mechanisms

Chapter Summary

- Humoral immunity is mediated by antibodies synthesized by B cells and secreted by plasma cells.
- Humoral immunity is the major defense mechanism against extracellular microbes and toxins.
- Most naturally occurring antigens are thymus-dependent: They require collaboration of TH and B cells.
- Contact between specific B and TH cells involves MHC class II/peptide presentation, costimulatory molecules (B7/CD28), CD40/CD40L binding, and cytokine production (IL-2, IL-4, IL-5,and IL-6).
- TH2 cells direct isotype switching by B cells, which changes the effector function of the antibody produced.
- Thymus-independent antigens, such as bacterial lipopolysaccharide, cross-link the receptors of B lymphocytes and cause them to proliferate and secrete IgM antibodies. These antigens *do not* create "immunologic memory."
- IgM is the first isotype of antibody that can be produced. It exists in serum as a pentamer held together by a joining (J) chain.
- The functions of IgM are as a monomer (receptor on B cells), antigen capture in the secondary lymphoid organs and as a pentamer in plasma, and activation of complement.
- TH2 activation of B lymphocytes causes intense proliferation in the germinal centers, and somatic hypermutation may cause slight variation in the shape of the idiotype. Clonal selection of the idiotype with the highest affinity for antigen results in "affinity maturation": a general improvement in the "goodness-of-fit" for the antigen as the immune response progresses.
- IgG is the major isotype produced after IgM. It exists in four subisotypes. It activates complement, opsonizes, mediates ADCC, and is actively transported across the placenta.
- IgA is the major isotype produced in the submucosa, colostrum, and breast milk. It is a dimer with a J chain holding it together. It functions in inhibiting the binding of substances to cells or mucosal surfaces. It does not activate complement or mediate opsonization.
- IgE is the antibody that binds to mast cells and is responsible for antihelminthic and allergic responses.
- Complement is a set of interacting serum proteins that enhance inflammation (C3a, C4a, C5a) and opsonization (C3b) and cause lysis of particulate substances (C5b-9).
- The alternative pathway of complement is activated by interaction with microbial surfaces.
- The classical pathway is activated by antigen–antibody complexes.
- Inappropriate activation of the complement cascade is controlled at the level of C1, C3, and C5.

Review Questions

1. An antibody preparation is being used in a laboratory protocol to study B lymphocytes. The preparation does not activate the cells or cause capping. It does not cause precipitation of its purified ligand, and it does not cause agglutination of latex beads covalently coupled to its ligand. Which of the following is the most likely antibody preparation?

   (A) Monoclonal anti-CD19 IgG
   (B) Monoclonal anti-CD56 IgG
   (C) Papain-treated anti-CD19 IgG
   (D) Papain-treated anti-CD56 IgG
   (E) Pepsin-treated anti-CD19 IgG
   (F) Pepsin-treated anti-CD56 IgG

2. IgM isohemagglutinins from an individual of blood group A are treated with pepsin. When the product of this reaction is added to group B erythrocytes, they will be

   (A) agglutinated
   (B) lysed
   (C) phagocytized
   (D) precipitated
   (E) unaffected

3. A 26-year-old obstetric patient becomes ill during the first trimester of pregnancy with fever and lymphadenopathy. She is found to have a rising titer of anti-*Toxoplasma gondii* antibodies. She delivers a full-term baby with no apparent signs of in utero infection. The best test to diagnose acute infection in the neonate would be a parasite-specific ELISA for which isotype of immunoglobulin?

   (A) IgA
   (B) IgD
   (C) IgE
   (D) IgG
   (E) IgM

4. A 4-year-old boy is evaluated for a possible immunologic deficiency. He has suffered repeated infections of mucosal-surface pathogens and has shown delayed development of protective responses to the standard childhood vaccinations. Immunoelectrophoresis of his serum demonstrates absence of a macroglobulin peak, and his sputum is devoid of secretory IgA. Normal numbers of B lymphocytes bearing monomeric IgM are found by flow cytometry, and serum levels of monomeric IgA, IgE, and each of the four subisotypes of IgG are normal. Which of the following deficiencies could account for these findings?

   (A) Absence of CD40
   (B) Absence of J chains
   (C) Absence of IL-4
   (D) Absence of Tdt
   (E) Absence of TH2 cells

5. A 56-year-old homeless, alcoholic, and febrile man is brought to the emergency department after a difficult night during which his coughing kept everyone at the shelter awake. On arrival his pulse is rapid, and his breathing is labored with diffuse rales. Endotracheal aspirates produce a mucopurulent discharge containing numerous gram-positive cocci in chains. His serum contains high titers of IgM antibodies specific for the polysaccharide capsule of *Streptococcus pneumoniae*. The effector mechanism most likely to act in concert with this early IgM production to clear infection is

   (A) ADCC
   (B) complement-mediated opsonization
   (C) cytotoxic T lymphocytes
   (D) LAK cells
   (E) NK cells

6. A 3-year-old boy has had several bouts with pneumonia. *Streptococcus pneumoniae* was isolated and identified in each of the cases. The child was treated with penicillin each time, and the condition resolved. He is now being evaluated for a potential immunologic deficiency. Serum electrophoresis shows age-normal values for all isotypes of immunoglobulin, but serum levels of some components of complement are depressed. Which of the following deficiencies could explain his problem?

   (A) C1
   (B) C2
   (C) C3
   (D) C4
   (E) C5

Answers and Explanations

1. **The correct answer is C.** The cell surface marker which is typically used to identify B lymphocytes is CD19. This is a component of the B-cell/signal transduction complex and thus will be found on all B cells. Treatment of IgG with papain yields two monovalent antigen binding (Fab) fragments and destroys the function of the Fc portion of the molecule. Immunoglobulin molecules that are disrupted this way lose their ability to cross-link the receptors on cells, to promote precipitation or agglutination, and to activate cells by providing a first stimulatory signal.

   Monoclonal anti-CD19 IgG (**choice A**) is a divalent antibody molecule that recognizes the signal transduction complex on B cells. Monoclonal antibodies can cross-link cell-surface receptors and cause capping, cell activation, and precipitation. Agglutination is usually accomplished using IgM because a very large molecule is needed to overcome the zeta potential (repulsive charge) of erythrocytes. If IgG is used, a second developing antibody must be added.

   Monoclonal anti-CD56 IgG (**choice B**) is a divalent antibody molecule that recognizes a molecule found on NK cells. Because both arms of the molecule are intact, it is capable of causing capping, cell activation, precipitation, and agglutination if a developing antiserum is added.

   Papain-treated anti-CD56 IgG (**choice D**) would not be used for the study of B lymphocytes because CD56 is a marker for NK cells.

   Pepsin-treated anti-CD19 IgG (**choice E**) is a divalent molecule possessing two Fab fragments joined together ($F[ab']_2$), and a fragmented Fc region. The $F(ab')_2$ portion of the antibody is capable of causing capping, cell activation, precipitation, and, with a developing antiserum, agglutination.

   Pepsin-treated anti-CD56 IgG (**choice F**) is a divalent molecule possessing two Fab fragments joined together ($F[ab']_2$) and a fragmented Fc region. Its specificity is for NK cells. Additionally, the $F(ab')_2$ portion of the antibody is capable of causing capping, cell activation, precipitation, and, with a developing antiserum, agglutination.

2. **The correct answer is A.** Isohemagglutinins are IgM antibodies that will agglutinate the RBCs of individuals with another blood type. They are believed to be made due to exposure to cross-reactive antigens found on the surface of normal gut flora organisms. Thus, a person of blood group A will produce isohemagglutinins that will agglutinate type B cells. If these antibodies are pretreated with pepsin, a divalent $F(ab')_2$ fragment and destruction of the Fc will result. A divalent fragment is capable of causing agglutination.

   Lysed (**choice B**) is not correct because it would require the integrity of the complement-binding regions of the IgM, which are found in the Fc, and the question stem does not provide complement in the mix.

   Phagocytized (**choice C**) is not correct because it would require an intact cell-binding region in the Fc, and the question stem does not provide phagocytic cells in the mix.

   Precipitated (**choice D**) would be the correct answer if the antigen in question were a soluble protein. Proteins precipitate when treated with specific antibodies, particles agglutinate. The two particles used in laboratory medicine are latex beads and erythrocytes. If neither of these is mentioned, then the student can assume that treatment would result in precipitation, not agglutination. Precipitation has exactly the same requirements as agglutination: a divalent antigen-binding molecule.

   Unaffected (**choice E**) would be the correct answer if papain had been used to treat the isohemagglutinins. Because papain produces two monovalent Fab fragments, these are incapable of cross-linking antigen (whether soluble protein or particle), so neither agglutination nor precipitation would be possible.

3. **The correct answer is E.** The only way to identify a neonatal infection serologically is by detection of pathogen-specific IgM antibodies. This is because the fetus receives IgG antibodies from the mother by active transport across the placenta. Because you cannot identify the source of the antibodies, IgG detection in the child can simply reflect this natural passive type of protection. Because IgM does not cross the placenta, any IgM detected in the neonate is being produced in the child and is reflective of a response to infection. In this way, all children born to HIV-infected mothers will be seropositive by both ELISA and Western blot, but only 20% will actually be infected in utero, even in the absence of antiviral therapy.

   IgA (**choice A**) does not usually begin to be produced by a child until one to two years after birth. At the end of the first year, most children have no more than 20% of adult values, so it would not be a useful diagnostic in the neonate. Additionally, because *Toxoplasma gondii* is an intracellular parasite, IgA would not be the most effective immune response in any individual.

   IgD (**choice B**) will be produced by an infected neonate along with IgM because of alternative RNA splicing, but this is not a useful diagnostic. IgD rarely reaches levels easily detected by serology, and the immunoglobulin has the shortest half-life of all the immunoglobulins. The function of secreted IgD, if any, is not clear, so it is not a useful serologic test.

   IgE (**choice C**) does not usually begin to be produced by a child until well into the second year after birth. Additionally, because *Toxoplasma gondii* is an intracellular parasite, IgE would not be the most effective immune response in any individual.

   IgG (**choice D**) is not a useful serologic test in a neonate because it is impossible to determine the origin of such molecules. Children infected in utero will begin to produce IgG due to isotype switching late in gestation, but because the placenta is actively transporting all maternal IgG into the fetus, it is not possible to distinguish whether the child is actually infected or simply passively protected using this technique.

4. **The correct answer is B.** IgM and secretory IgA are similar in that they are held together by a J chain synthesized by the B cell or plasma cell. Without the presence of the J chain, IgM would exist only in monomeric form, and the macroglobulin peak would be absent on electrophoresis. Because pentameric IgM is important for capturing newly introduced foreign antigen and thus beginning the immune response, the child is delayed in his development of protective responses to vaccination. Because secretory IgA is a dimer that protects the mucosal surfaces, such a child would be especially susceptible to infectious agents crossing the mucosal surfaces.

   Absence of CD40 (**choice A**) would affect the production of IgG, IgA, and IgE, but would not prevent macroglobulin synthesis. Indeed, most patients with this defect have hypermacroglobulinemia because the CD40/CD40L interaction is necessary for isotype switching.

   Absence of IL-4 (**choice C**) would cause problems with the ability to produce IgG, IgA, and IgE. This cytokine, produced by TH2 cells, is necessary for the differentiation and development of most antibody responses other than IgM. Thus, IgM levels either would not be affected or would be increased in a compensatory fashion.

   Absence of Tdt (**choice D**) would cause problems with the patient's ability to perform the genetic rearrangements necessary to form the idiotype of the antibody molecule. They would not affect the isotype of antibody produced.

   Absence of TH2 cells (**choice E**) would affect the production of IgG, IgA, and IgE, but would not affect IgM production.

5. **The correct answer is B.** One of the most effective protective responses to infections with extracellular, encapsulated bacteria, such as *Streptococcus pneumoniae*, is complement-mediated opsonization. Because IgM is the most effective antibody at activating complement, generation of C3b fragments during this process coats the bacteria and makes them more susceptible to ingestion and intracellular killing by cells of the phagocytic system.

   ADCC (**choice A**), or antibody-dependent cell-mediated cytotoxicity, is a mechanism by which NK cells, neutrophils, macrophages, and eosinophils can use their Fc receptor to bind specific antibody and target an agent for lysis. No cells have Fc receptors for IgM, so this is not a mechanism that could act in concert with early IgM production.

   Cytotoxic T lymphocytes (**choice C**) identify altered-self/MHC class I molecule conjugates on the surfaces of cells that are malignantly transformed or infected with intracellular pathogens. They are not a protective mechanism that acts in concert with any antibody molecule.

   LAK cells (**choice D**), or lymphokine-activated killer cells, are NK cells that have been stimulated in vitro with cytokines that enhance their killing activity. These cells have a function in early surveillance against altered-self cells, but are not believed to play a role in protection against extracellular pathogens, such as this one.

   NK cells (**choice E**) are members of the innate immune system and are believed to play a role in surveillance against tumor cells and other altered-self cells that fail to express MHC class I antigens on their surfaces. They would not act in concert with IgM production, and they would not be effective against an extracellular pathogen, such as this one.

6. **The correct answer is C.** The component of complement that is most important in clearance of extracellular pathogens such as *Streptococcus pneumoniae* is C3b. This fragment acts as an opsonin and enhances the ingestion and intracellular killing of the bacteria by phagocytic cells.

   C1 (**choice A**) is the first component of the complement cascade activated in the classic pathway. Although it is critical to initiating those events that can culminate in the production of the membrane attack complex, it is not the most important component for the clearance of infections such as this one.

   C2 (**choice B**) is the third component of the complement cascade activated in the classic pathway. Although it is critical to initiating those events that can culminate in the production of the membrane attack complex, it is not the most important component for the clearance of infections such as this one.

   C4 (**choice D**) is the second component of the complement cascade activated during the classic pathway. Although it is critical to initiating those events that can culminate in the production of the membrane attack complex, it is not the most important component for the clearance of infections such as this one.

   C5 (**choice E**) is the fifth component of the complement cascade activated during the classic pathway and the first step in the formation of the membrane attack complex (C5b–9). It is not the most important component for the clearance of infections such as this one.

The Generation of Cell-Mediated Effector Mechanisms

8

What the USMLE Requires You to Know

- The biologic function of cell-mediated immunity (CMI)
- The effector cells of CMI, their targets, and mechanisms of killing
- The means of regulation of CMI responses

The cell-mediated arm of the immune response (CMI) is designed to identify and eradicate antigenic stimuli that arise from **inside** the cells of the body. This occurs when cells of the host become infected with intracellular pathogens, such as viruses, some parasites, and bacteria, or when malignant transformation causes cells to express aberrant surface molecules. In such cases, TH1 cells primed in the lymph nodes and spleen serve to provide the cytokine stimuli to activate the three potential effector cells to destroy the infected or altered cells: **cytotoxic CD8+ T lymphocytes (CTLs), macrophages,** and **NK cells.**

In A Nutshell

- CMI protects against intracellular pathogens.
- TH1 cells stimulate:
  - Macrophages
  - CTLs
  - NK cells

One example of a cell-mediated effector mechanism that is enhanced by the action of TH1 cells is macrophage killing. This is a critical protective mechanism in the defense against organisms invading macrophages and attempting to live there (mycobacteria, *Leishmania*) or in the case where phagocytosed microbes have protective mechanisms that make them resistant to intracellular digestion (*Listeria*). In CMI against phagocytosed microbes, the specificity of the response arises from T cells, but the actual effector function is mediated by the phagocytes. This provides an important link between the adaptive and innate immune responses, and in essence, converts phagocytes into agents of the adaptive immune response. The most important cytokine elaborated by TH1 cells and CD8+ T lymphocytes to enhance the microbicidal capabilities of phagocytes is IFN-γ. In addition, production of TNF-α and TNF-β by T cells enhances inflammation and provides other stimuli that activate phagocytic cells. Macrophages and other phagocytes kill microbes intracellularly (as discussed in Chapter 6) in contrast to the mechanism observed with CTLs and NK cells.

In A Nutshell

- Macrophages kill intracellularly.
- Killing is enhanced by IFN-γ, TNF-α, and TNF-β.
- The DTH skin test measures TH1 function.

When TH1 cytokines activate macrophages and cause tissue damage, the result is **delayed-type hypersensitivity (DTH)** (*see* Chapter 13). Assay of DTH by skin testing is often used as a measure of the patient's ability to mount a CMI response (e.g., Mantoux test, Lepromin test).

The CTL recognizes the cell it will ultimately kill by interaction between its TCR and MHC class I antigens on the surface of the target cell. If the cell in question is performing normal functions and therefore producing normal "self" peptides, there should be no CD8+ T cells that have a complementary TCR structure. If the cell is infected with an intracellular parasite or is expressing neoantigens reflective of tumor transformation, however, some small proportion of those CD8+ cells generated from the thymus should be capable of binding their TCRs to this MHC class I/non-self peptide combination. Unfortunately, because of the extreme polymorphism of the HLA system in humans, when tissues are transplanted between nonidentical individuals,

cells of the transplant are often targeted by CTLs as abnormal. In spite of the fact that they may only be presenting normal cellular peptides, in these cases the HLA molecules themselves are different enough to turn on the system (*see* Chapter 14).

Clinical Correlate

MHC Class I Deficiency

A recessively inherited deficiency in the production of MHC class I molecules has been described in rare individuals. Some of these cases result from the failure of TAP molecules to transport MHC I molecules to the surface of the cell, and others are due to the production of aberrant or nonfunctional MHC I molecules themselves. These patients, as anticipated, suffer from profound deficiencies of CD8+ T cells, although numbers of CD4+ T cells are normal. This is because MHC class I expression in the thymus is essential to the development of committed CD8+ cells. These individuals are susceptible to multiple, recurrent viral infections, but interestingly, not all viral infections appear to be involved. It may be that they are able to compensate in the case of some specific viral infections, by using γ-δ T cells (which are not dependent on thymic education and the expression of MHC) or NK cells to control those infections, whereas other viruses require killing by CD8+ cells alone. Patients with this defect will possess a normal ability to mount CD4+ cell responses such as DTH and antibody production, and indeed their antibody responses may be higher than usual, presumably because of the absence of inhibitory feedback signals (IFN-γ) from CD8+ cells directed toward TH2 responses. Thus, they are not very efficient at terminating antibody responses and tend to overproduce them as a result.

In A Nutshell

CTL stimulation requires:

- Non-self peptide/class I MHC
- CD28-B7
- IL-2

CTLs are capable of differentiation and cloning by themselves in the presence of the appropriate non-self peptide/class I MHC antigen stimulus, but are much more effective in so doing if they are assisted by signals from APCs or TH1 cells. CD8+ T cells express CD28 molecules, which can be bound by the B7 complementary molecule on the surface of APCs, which simultaneously express the appropriate MHC class I/peptide combination. This provides a costimulatory signal that stimulates CTL differentiation and cloning. The TH1 cell secretes IL-2 similarly acting on CD8+ cells to enhance their differentiation and cloning.

CTLs kill their target by the delivery of toxic granule contents that induce the apoptosis of the cell to which they attach. This process occurs in four phases:

- **Attachment** to the target (mediated by TCR, CD8, and LFA-1 integrin)
- **Activation** (cytoskeletal rearrangement to concentrate granules against attached target)
- **Exocytosis** of granule contents (perforin and granzymes)
- **Detachment** from the target

In A Nutshell

CTLs kill by:

- Perforins
- Cytokines
- Granzymes
- Fas/FasL

The actual production of apoptosis in the target may be mediated in distinct fashions. First, **perforin** present in the CTL granules creates pores in the membrane of the target cell through which **granzymes** (serine proteases) enter the target, inducing the activation of caspases, which in turn induces apoptosis. Second, cytokines such as IFN-γ with TNF-α or TNF-β can also induce apoptosis. Furthermore, activated CTLs express a membrane protein called **Fas ligand** (FasL), which may bind to its complementary structure on the target, Fas. When this occurs, caspases are induced and apoptosis results.

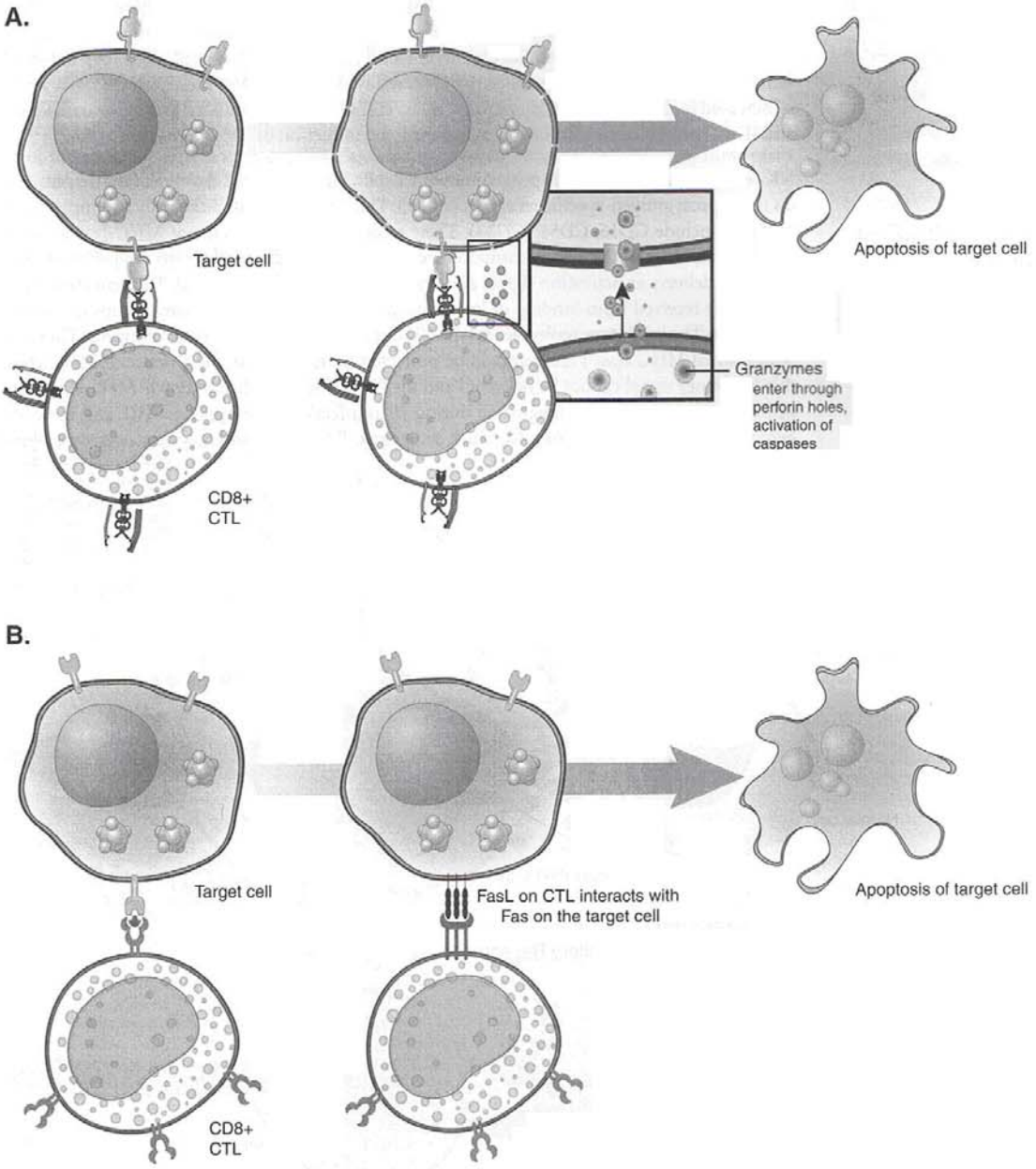


Figure II-8-1. Mechanisms of Cytotoxic T-Cell Killing. ***A:*** **Perforin and Granzymes, and** ***B:*** **Fas/Fas Ligand Interaction**

In A Nutshell

NK Cells

- Kill tumor cells and virus-infected cells
- Kill by granzymes and perforin
- Enhanced by IFN-α, IFN-β, and IL-12
- Inhibited by MHC class I
- Counted with CD16 and CD56

Another cell-mediated effector mechanism enhanced by the action of TH1 cells is NK cell killing. NK cells are the only lymphocyte members of the innate branch of the immune response. They exhibit the capacity to kill cells infected with some viruses and tumor cells, and they kill via the same mechanisms of inducing apoptosis observed with CTLs (granzymes, perforin). NK activity is increased in the presence of interferons (IFNs) α and β (IFNs stimulated during viral infections) and IL-12 (produced by phagocytic cells during the induction of TH1 responses).

NK cells share a common early progenitor with T cells, but they do not develop in the thymus. They do not express antigen-specific receptors or CD3. The markers are used clinically to enumerate NK cells and include CD16, CD56 (CD3–). Their recognition of targets is not MHC-restricted, and their activity does not generate immunologic memory. NK cells employ two categories of receptors: One delivers an activation signal, and one delivers an inhibitory signal. The activation signals seem to be received from binding of lectins possibly conserved among many groups of common pathogens. The inhibitory molecules on the NK cell seem to bind MHC class I antigens: Thus, a cell with normal MHC class I antigens will be protected from killing. In the absence of MHC class I, this inhibitory signal cannot be delivered and the NK cell will kill the target cell. MHC class I antigen expression may be downregulated during virus infections (*see* Appendix IV), and these antigens may be lost among tumor cells, which are genetically unstable and may delete portions of their genome (*see* Chapter 15).

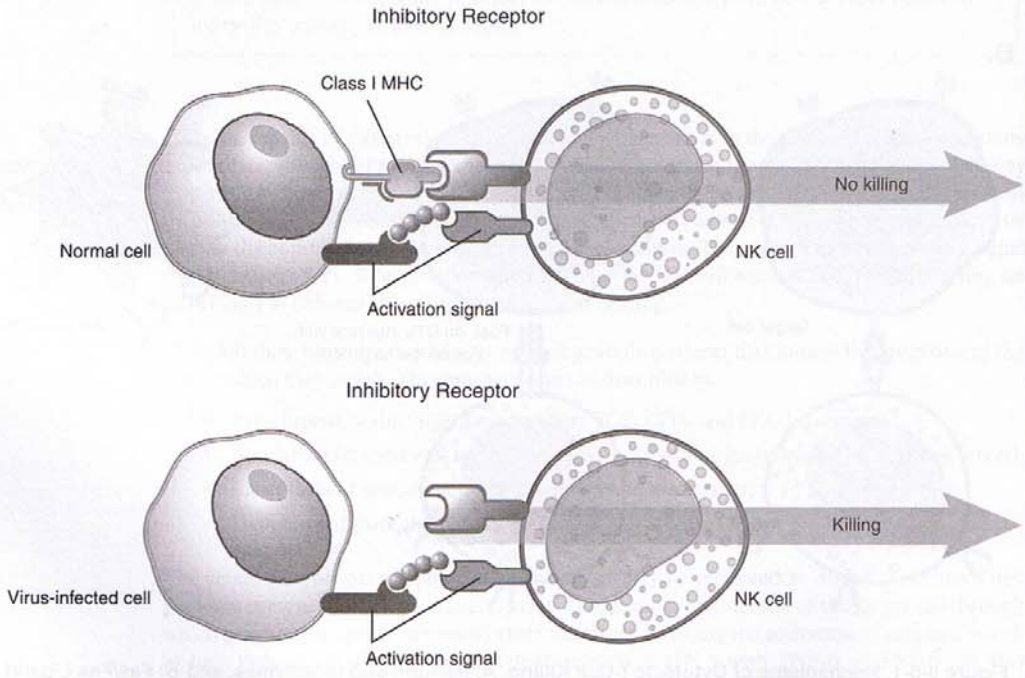


Figure II-8-2. Activation of NK Cells

A final mechanism of cell-mediated cytotoxicity that bridges humoral and cell-mediated effector systems in the body is **antibody-dependent cell-mediated cytotoxicity (ADCC)**. A number of cells with cytotoxic potential (NK cells, macrophages, monocytes, neutrophils, and eosinophils) have membrane receptors for the Fc region of IgG. When IgG is specifically bound to a target cell, the cytotoxic cells can bind to the free Fc "tail" and subsequently cause lysis of the target cell. Although these effectors are not specific for antigen, the specificity of the idiotype of the antibody directs their cytotoxicity. The mechanism of target cell killing in these cases may involve

- Lytic enzymes
- Tumor necrosis factor
- Perforin

In A Nutshell

ADCC

- NK cells
- Macrophages and monocytes
- Neutrophils
- Eosinophils
- Target recognition via IgG
- Killing by lytic enzymes, TNF, and perforin

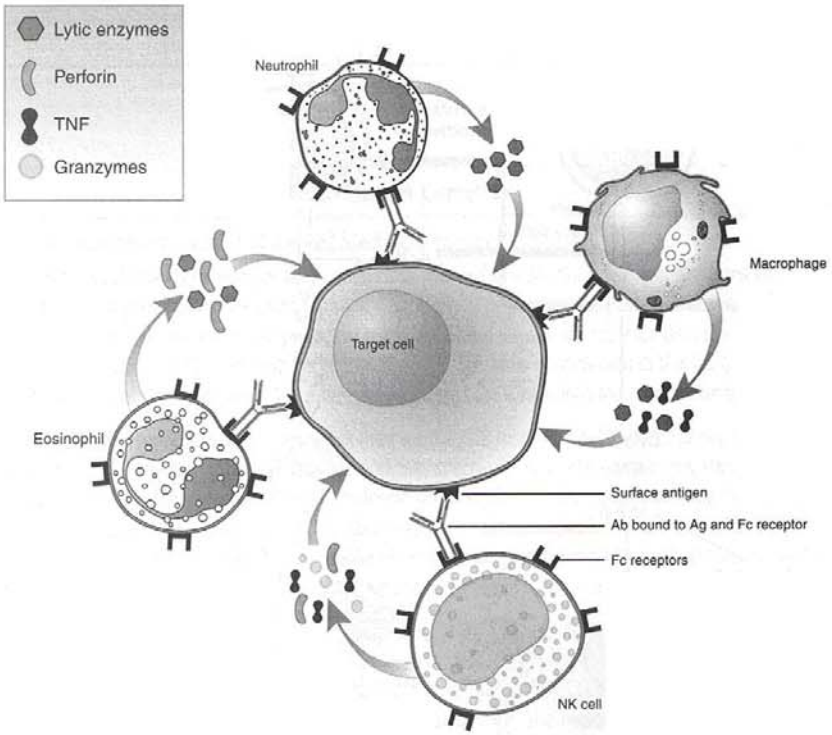


Figure II-8-3. Antibody-Dependent Cell-Mediated Cytotoxicity

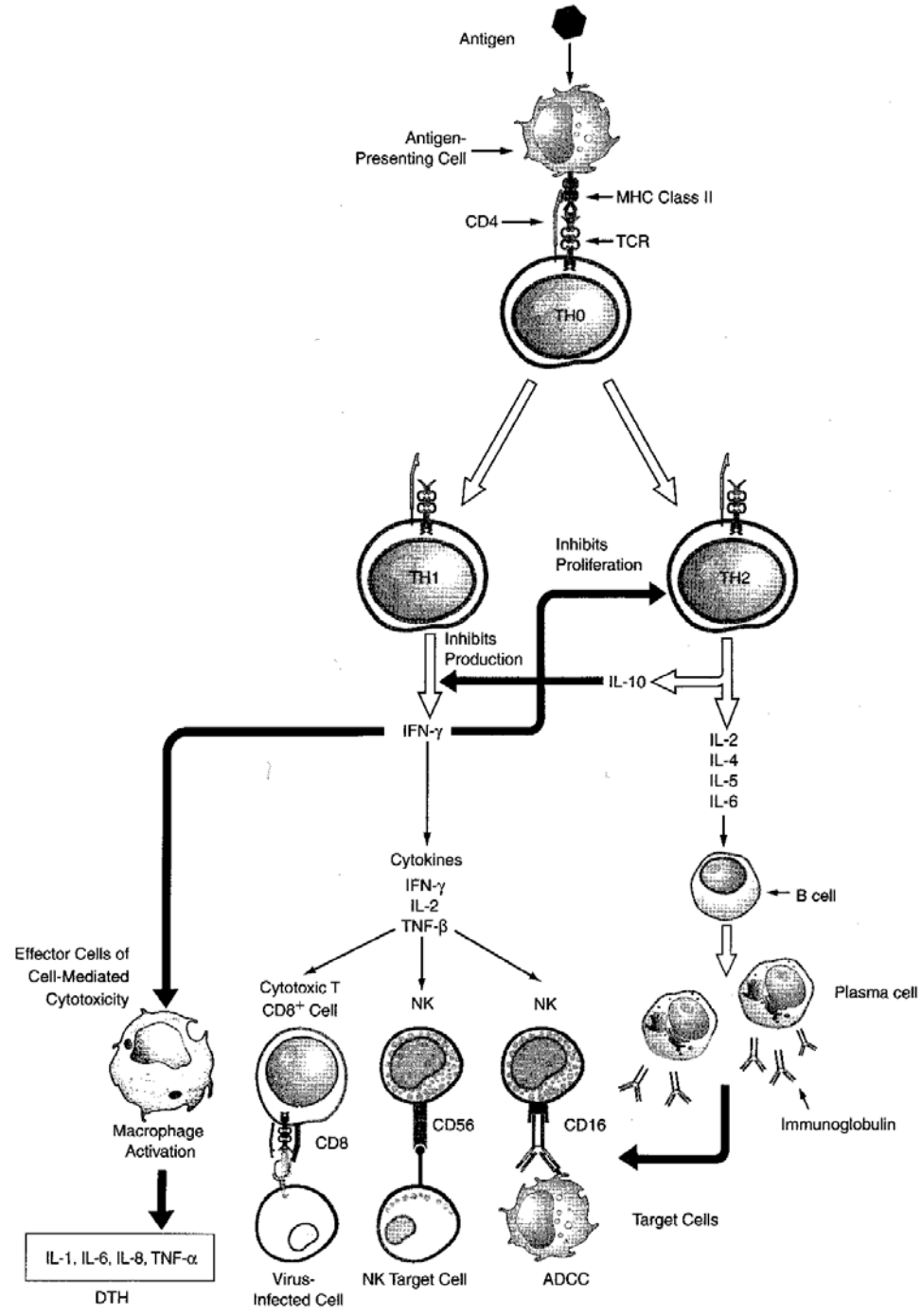


Figure II-8-4. Overview of Cell-Mediated Immunity

Table II-8-1. Effector Cells in Cell-Mediated Immunity

Effector Cell	CD Markers	Antigen Recognition	MHC Recognition Required for Killing	Effector Molecules
CTL	TCR, CD3, CD8, CD2	Specific, TCR	Yes, class I	Perforin, granzymes, cytokines (TNF-β, IFN-γ)
NK cell	CD16, CD56, CD2	ADCC: specific by IgG; otherwise, recognizes lectins	No, MHC I recognition inhibits	Perforin, granzymes, cytokines (TNF-β, IFN-γ)
Macrophage	CD14	Nonspecific	No	TNF-α, enzymes, NO, oxygen radicals

Clinical Correlate

Viral Strategies for Evasion of the Cell-Mediated Immune Response

In the struggle for survival inside the human host, viruses have developed several strategies for evasion of protective cell-mediated immune responses. In viruses that replicate quickly, as do most RNA viruses, CTLs are the major protective response. These viruses will replicate until the TH1 response turns on CTLs and destroys all infected cells. The virus is eradicated in this host, but the rate of viral replication has ensured that a new nonimmune host is now infected to continue the cycle.

In viruses that replicate slowly and cannot out-race the CTL response, a different strategy is necessary. Many such viruses block host cell protein synthesis, specifically MHC class I synthesis, transport, and expression (see Appendix IV). With this molecule downregulated, infected cells become "immune" to CTL recognition and killing. The downregulation of class I MHC, however, makes most of these viruses susceptible to the second cell-mediated immune mechanism, NK killing. Therefore, in most cases, the conjunction of CTL and NK mechanisms is sufficient to eradicate viral infections.

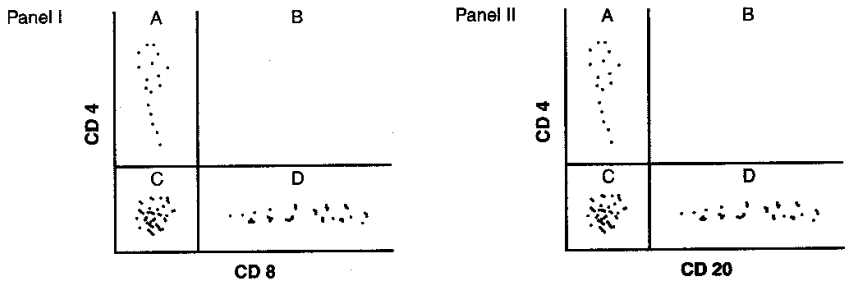
The exception to this rule is cytomegalovirus (CMV). CMV downregulates the class I MHC molecule like many other viruses, but also produces a "decoy" MHC class I–like molecule. This virally encoded decoy molecule is too different from the host's to be recognized by CTLs, but it is sufficient to fool the NK cell, so CMV escapes both of these killing mechanisms. The reason humans are not all overwhelmed by this pathogen is that CMV can be successfully killed by the third cell-mediated immune response, ADCC. IgG molecules with specificity for the surface-expressed CMV antigens, such as the decoy molecule, will bind to virus-infected cells and be recognized by Fc receptor–bearing effector cells, like NK cells, which will then kill the target.

Chapter Summary

- The cell-mediated immune response protects against intracellular pathogens.
- TH1 cells activate macrophages, CTLs, and NK cells.
- Macrophages kill intracellularly in response to TNF-α, TNF-β, and IFN-γ activation.
- The DTH skin test measures TH1 function.
- CTLs kill targets wearing MHC class I/altered-self peptides, using perforin, granzymes, and Fas ligand.
- CTLs are stimulated by CD28/B7 interaction and IL-2 from TH1 cells.
- NK cells kill tumor and virus-infected cells using granzymes and perforin.
- NK cells are stimulated by IFN-α, IFN-β, and IL-12, and kill targets lacking MHC I.

Review Questions

1. An experimental treatment for melanoma involves in vitro stimulation of tumor-specific killer cells with tumor cells transfected with a gene for production of altered-self MHC class I molecules. As a first step, peripheral blood leukocytes from the patient are incubated with fluorescent-labeled antibodies against CD4, CD8, and CD20. The cells are then subjected to flow cytometry and separated into different populations based on their expression of these cell surface markers. In which quadrant would you find the cell subpopulation most likely to produce a beneficial anti-tumor response in this protocol?



(A) Panel I, quadrant A
(B) Panel I, quadrant B
(C) Panel I, quadrant C
(D) Panel I, quadrant D
(E) Panel II, quadrant A
(F) Panel II, quadrant B
(G) Panel II, quadrant C
(H) Panel II, quadrant D

2. A 62-year-old accountant develops a solid tumor that is unresponsive to chemotherapy. He elects to participate in an experimental treatment protocol to stimulate his own immune effector cells to recognize and kill the malignant cells. The tumor cells are found to have no expression of MHC class I antigens. Which of the following in vitro treatments of his tumor cells is likely to stimulate the most effective immune response when reinfused into the patient?

(A) IFN-γ
(B) IL-2
(C) IL-8
(D) IL-10
(E) TNF-β

3. *Toxoplasma gondii* is an intracellular parasite that lives inside phagocytic and nonphagocytic cells by generating its own intracellular vesicle. This may allow it to avoid recognition and killing by CD8+ lymphocytes, which require the presentation of foreign peptides transported into the endoplasmic reticulum and loaded onto MHC molecules that have

   (A) a β_2 domain instead of a β_2 microglobulin
   (B) invariant chains
   (C) a peptide-binding groove
   (D) a single transmembrane domain
   (E) two similar chains

4. Before 1960, children with enlarged thymus glands were frequently irradiated to functionally ablate this organ, whose role was not yet known. Over the lifetime of such individuals, which of the following conditions was likely to develop?

   (A) Depressed immune surveillance of tumors
   (B) Depressed oxygen-dependent killing by neutrophils
   (C) Depressed primary response to soluble antigens
   (D) Increased cellularity of lymph node paracortical areas
   (E) Increased tendency toward atopy

5. A 42-year-old Nigerian man who is in the United States visiting with his brother comes into the hospital clinic. He complains of several months of weight loss, night sweats, mild sputum production, and the spitting up of blood. You run a PPD skin test and the results are positive. What can you conclude from this result?

   (A) A cell-mediated immune response has occurred
   (B) A humoral immune response has occurred
   (C) The B-cell system is functional
   (D) The B- and T-cell systems are functional
   (E) The neutrophilic phagocyte system is functional

Answers and Explanations

1. **The correct answer is D.** To generate tumor-specific killer cells in vitro that would kill tumor cells transfected with an altered-self MHC class I gene, one would need to start with potential killer cells that use MHC I as a stimulatory signal. The only cytotoxic cell in the body that meets these criteria is the cytotoxic T lymphocyte (CTL). To determine which quadrant of the two figures would contain CTLs, the student needs to know how to interpret the data generated by the flow cytometer. In each case, the instrument plots the fluorescence of a cell passing through it as an individual dot on the histogram. So in panel I, increasing levels of fluorescence with antibody to CD8 are plotted as one moves to the right, and increasing levels of fluorescence with antibody to CD4 are plotted as one moves upward. Thus, the cells most strongly positive with CD8 are found the farthest to the right in quadrant D of panel I.

   Panel I, quadrant A (**choice A**) would contain cells that are CD4+ and CD8–. These would be helper cells, and they would not be cytotoxic to transfected tumor cells.

   Panel I, quadrant B (**choice B**) would contain cells that are double-positive for CD4 and CD8. These cells would be found as immature thymocytes in the thymus and not in the blood; thus, there are no double-labeled cells shown in this quadrant.

   Panel I, quadrant C (**choice C**) contains the cells that have only background levels of fluorescence with antibodies to CD4 and CD8. These would be nonhelper, noncytotoxic cells, so they could be B lymphocytes, NK cells, or any other peripheral blood leukocyte.

   Panel II, quadrant A (**choice E**) would contain cells which are strongly CD4+ and CD20–. These are helper T lymphocytes.

   Panel II, quadrant B (**choice F**) would contain cells positive for CD4 and CD20. Because CD4 is a TH cell marker, and CD20 is a B-cell marker, such cells do not exist and thus this quadrant is empty.

   Panel II, quadrant C (**choice G**) contains the cells that have only background levels of fluorescence with antibodies to CD4 and CD20. These would be nonhelper, non-B cells, so they could be cytotoxic T lymphocytes, NK cells, or any other peripheral blood leukocyte. Although this quadrant clearly contains some of the cytotoxic cells that this question asks about, there are other cells present, so this is not the best answer.

   Panel II, quadrant D (**choice H**) contains the cells strongly positive for CD20 and negative for CD4. These are B lymphocytes.

2. **The correct answer is A.** The killer cells cytotoxic to targets lacking MHC class I antigens are NK cells. These cells are members of the innate immune response, and as such their response is not enhanced over time. The most specific, inducible cytotoxic cells in the body are cytotoxic T lymphocytes (CTLs), which depend on MHC class I recognition of their target. Because this question asks how the tumor cells can be altered to make them better stimulators of an immune response, one approach would be to increase their expression of MHC class I molecules. This can be accomplished by treatment of the tumor cells with interferon (IFN)-γ. IFN-γ increases expression of both classes I and II MHC products on cells.

   IL-2 (**choice B**) is a product of TH1 lymphocytes and induces proliferation of antigen-primed TH and cytotoxic T cells. It also supports their long-term growth. It would not have an effect on this patient's tumor cells.

   IL-8 (**choice C**) is a product of macrophages and endothelial cells and acts on neutrophils to cause their chemotaxis and extravasation into tissues. It would not have an effect on this patient's tumor cells.

   IL-10 (**choice D**) is a product of TH2 cells and acts on macrophages to suppress their cytokine production. It therefore indirectly reduces cytokine production by TH1 cells and

dampens the activation of the cell-mediated arm of the immune response. It would not have an effect on this patient's tumor cells.

TNF-β (**choice E**) is a product of macrophages and NK cells and acts on tumor cells to cause direct cytotoxicity. It acts on inflammatory cells to induce cytokine secretion and causes the cachexia associated with chronic inflammation. It would not have an effect on this patient's tumor cells.

3. **The correct answer is D.** CD8+ lymphocytes, or cytotoxic T lymphocytes recognize their target cells by binding to MHC class I molecules containing altered-self peptides. The class I molecule is a two-chain structure, with one long α chain that passes through the cellular membrane and a shorter chain called β_2 microglobulin that becomes associated with the α chain.

   A β_2 domain instead of a β_2 microglobulin (**choice A**) describes the class II MHC molecule. It is loaded with peptides by the endosomal (exogenous) pathway and is recognized by CD4+ T cells.

   Invariant chains (**choice B**) are found blocking the peptide-binding groove of the class II MHC molecule immediately after synthesis. These chains are digested away when the class II MHC is exposed to the contents of the phagocytic vesicles of macrophages, and the groove is loaded with peptides from the ingested particle.

   A peptide-binding groove (**choice C**) would be found in both class I and II MHC molecules and is therefore not the best answer.

   Two similar chains (**choice E**) would be found in the class II MHC molecule. It is composed of an α and a β chain of similar lengths, both of which have transmembrane domains. The class II MHC molecule is loaded with peptides by the endosomal (exogenous) pathway and is recognized by CD4+ T cells.

4. **The correct answer is A.** Although the ablation of the thymus in early childhood will ultimately have far-reaching consequences in the development of many immune responses, the immune surveillance of tumors is performed only by cytotoxic T cells and NK cells, and thus would be profoundly affected by this treatment. Other parameters that could be depressed include immune responses to intracellular pathogens and secondary antibody responses.

   Depressed oxygen-dependent killing by neutrophils (**choice B**) would not be expected in this case because neutrophils are components of the innate immune response and function in the absence of T-cell help.

   Depressed primary response to soluble antigens (**choice C**) would not be expected in this case because the IgM response to many antigens is T-cell independent. It is class switching that would be impossible without T-cell help.

   Increased cellularity of lymph node paracortical areas (**choice D**) would not be expected in this case because the paracortex of lymph nodes is a T-cell area. Therefore, following thymic irradiation, decreased cellularity of these regions would occur.

   Increased tendency toward atopy (**choice E**) would not be expected in this case because atopic allergies are those that involve IgE antibodies and mast cells. IgE cannot be produced without T-cell help, so athymic individuals will have decreased tendency toward atopy.

5. **The correct answer is A.** The Mantoux test, or tuberculin test (or simply the TB skin test), is the classic clinical demonstration of the function of the delayed-type hypersensitivity response. This is a cell-mediated reaction caused by sensitization of TH1 cells and demonstrated by the influx and activation of macrophages in response to the cytokines that they elaborate.

That a humoral immune response has occurred (**choice B**) is not true. Antibodies are not involved in the production of a DTH response, and they are not important products during infections with most intracellular pathogens.

That the B-cell system is functional (**choice C**) is not true. B cells do not play a role in the DTH response, and they do not play a major role in defense during infections with most intracellular pathogens.

That the B- and T-cell systems are functional (**choice D**) is not true. The DTH response certainly demonstrates that the TH1 response is functional, but it says nothing about the function of B cells.

That the neutrophilic phagocyte system is functional (**choice E**) is not true. Neutrophils do not play a role in the elicitation of the DTH response.

The Generation of Immunologic Memory

9

What the USMLE Requires You to Know

- The characteristics of memory B and T lymphocytes
- The recirculation patterns of memory B and T lymphocytes
- The characteristics and function of secretory IgA
- The comparative attributes of the primary and secondary immune responses

As long as foreign antigen is present in the system, the activation, proliferation, and differentiation of lymphocytes into effector cells will continue in the secondary lymphoid organs. As the effector mechanisms generated are successful in destroying or causing clearance of the invader, however, the system will slowly return to its baseline quiescent state, and **immunologic memory** will be generated. This is important because it avoids expending energy on the generation of cells and molecules that are no longer needed and may be potentially harmful in the absence of the invading stimulus. It also "resets" the baseline homeostatic function of the immunologic organs so that they can efficiently respond to new and emerging challenges.

In A Nutshell

As pathogens are eliminated, immunologic memory is generated.

Both B- and T-lymphocyte populations will respond to a primary antigenic challenge by the production of long-lived memory cells. In B lymphocytes, this is primarily accomplished by the fact that their differentiation into plasma cells is antigen-dependent, and as that antigen disappears, the stimulus for that differentiation is removed. Plasma cells, which function only as factories for immunoglobulin synthesis, are relatively short-lived (two weeks) and as they die and are not replaced from the differentiating B-cell pool, the response wanes. Memory B lymphocytes differ from naive B cells in that they have undergone isotype switching; will bear membrane immunoglobulin of IgG, IgA, or IgE isotype; and enter a resting stage of the cell cycle.

In A Nutshell

Memory B cells have surface IgG, IgA, or IgE.

The body's mechanism of dampening T-cell activity after the primary immune response is more active, presumably because the cytokines they produce can have harmful effects if they are generated unnecessarily. These cells no longer benefit the host and can be potentially harmful, so apoptosis is induced in all but a few, which will become quiescent in a resting stage of the cell cycle. This is called **activation-induced cell death (AICD)** and is mediated through the Fas pathway. In this way, trimerization of the Fas molecule expressed on the surface of activated T cells with the Fas ligand molecule on neighboring cells initiates a signal-transduction cascade that leads to apoptosis of the Fas-bearing cell.

In A Nutshell

AICD removes activated T cells after the primary response.

Although the memory cells generated during the waning stages of the primary immune response are small and relatively quiescent, they exhibit high-level expression of adhesion molecules, which will help them to recirculate throughout the body and home to areas of new antigen introduction. In this way, a response that was initiated in a single draining lymph node will become generalized throughout the body and available to initiate a rapid and powerful secondary response if that challenge is reintroduced.

In A Nutshell

Memory cells home to inflamed tissues.

Table II-9-1. Characteristics of Naive, Effector, and Memory Lymphocytes

	Naive Lymphocytes	Activated or Effector Lymphocytes	Memory Lymphocytes
T lymphocytes			
Migration	To peripheral lymph nodes	To inflamed tissues	To inflamed tissues or mucosa
Effector functions	None	Cytokine secretion, cytotoxicity	None
Cell cycling	Absent	Present	+/–
IL-2 receptor	Low	High	Low
B lymphocytes			
Isotype of membrane Ig	IgM and IgD	IgG, IgA, or IgE	IgG, IgA, or IgE
Affinity of Ig	Low	Increasing	High
Effector function	None	Antibody secretion	None
Morphology	Small, little cytoplasm	Large, more cytoplasm	Small

In A Nutshell

Memory cells return to the tissue where they first encountered antigen.

Clinical Correlate

Dissemination of Immunologic Memory

The vaccine used by the military against adenovirus types 4 and 7 is an enteric-coated, live, nonattenuated virus preparation. This vaccine produces an asymptomatic intestinal infection and thereby induces mucosal IgA memory cells. These cells then populate the mucosal immune system throughout the body. Vaccine recipients are thus protected against adenovirus acquired by aerosol, which could otherwise produce pneumonia.

The trafficking patterns of memory lymphocytes are different than those of either naive or effector lymphocytes. As discussed in Chapter 4, naive cells tend to home to regions of the secondary lymphoid organs specific for their cell type (T cells to paracortical areas, etc.). Effector cells tend to home to areas of active inflammation because of their expression of cell adhesion molecules such as LFA-1. Memory cells tend to home in a tissue-specific fashion, presumably returning to the type of tissue in which they first encountered antigen. In this way, some memory cells express adhesion molecules that direct them to a protective location along the digestive tract, whereas others express different adhesion molecules that tend to direct them to protective sites in the dermis of the skin.

When an antigen is introduced into the system a second time, the response of lymphocytes is accelerated and the result amplified over that of the primary immune response. The increased speed of response is due to the presence of the memory-cell progeny of the first response throughout the body, and the increased amplitude of effector production is due the fact that activation and cloning now begin from a much larger pool of respondents.

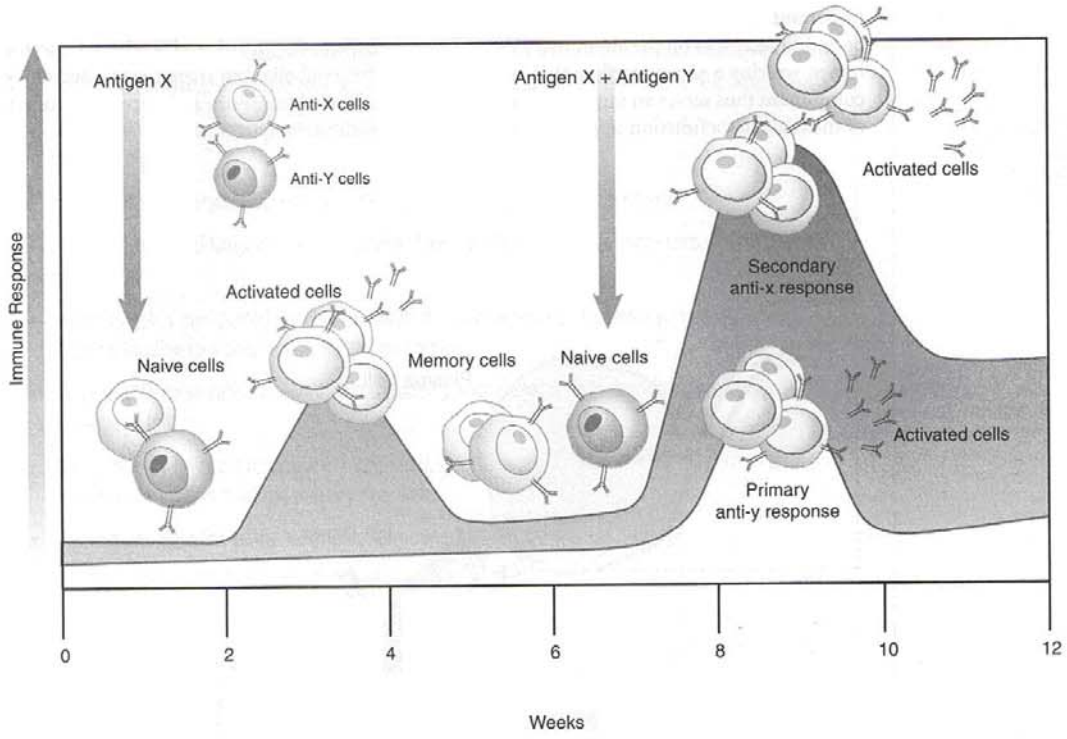


Figure II-9-1. The Primary and Secondary Immune Responses

Table II-9-2. Comparison of the Primary and Secondary Immune Responses

Feature	Primary Response	Secondary Response
Time lag after immunization	5–10 days	1–3 days
Peak response	Small	Large
Antibody isotype	IgM, then IgG	Increasing IgG, IgA, or IgE
Antibody affinity	Variable to low	High (affinity maturation)
Inducing agent	All immunogens	Protein antigens
Immunization protocol	High dose of antigen (often with adjuvant)	Low dose of antigen (often without adjuvant)

The homing of specific memory cells to epithelial and mucosal surfaces leads to the production of specialized lymphoid aggregations along these barriers. Collectively referred to as **mucosal-associated lymphoid tissues**, or **MALT**, they include the tonsils and Peyer patches, as well as numerous less well-organized lymphoid accumulations in the lamina propria. TH2 cells in these sites are dedicated to providing help for class switching to IgA. Most IgA-secreting B lymphocytes and plasma cells in the body will be found in these locations. **Secretory IgA** (that which is released across the mucosa of the respiratory, digestive, and urogenital tracts) differs from serum IgA in an

In A Nutshell

- MALT contains TH2 cells assisting IgA production.
- Secretory IgA is a dimer with secretory component.
- Secretory component:
  - Transepithelial transport
  - Protection from proteolytic cleavage

important fashion. As the IgA dimer is produced by plasma cells and B lymphocytes, it becomes bound to receptors on the abluminal side of the epithelia, is endocytosed, and is released into the lumen wearing a secretory piece that is the residue of the epithelial-cell receptor. The **secretory component** thus serves an important function in transepithelial transport, and once in the lumen of the tract, has a function in protecting the molecule from proteolytic cleavage.

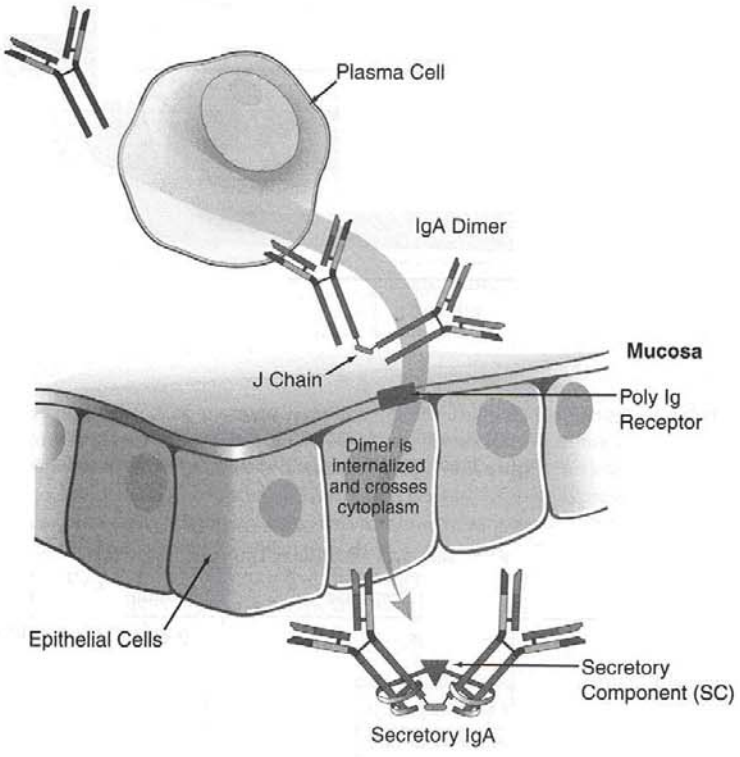


Figure II-9-2. Secretory IgA

Chapter Summary

- Immunologic memory is generated as pathogens are eliminated by the immune response.
- Long-lived B and T memory cells are generated and recirculate through the body in surveillance for the antigen.
- Memory B lymphocytes express IgG, IgA, or IgE molecules as antigen receptors.
- Mucosal-associated lymphoid tissues (tonsils, Peyer patches) are the primary sites of TH2 help for IgA production.
- Secretory IgA is transported into the lumen of the gastrointestinal, respiratory, or genitourinary tracts by binding to a polyimmunoglobulin receptor.
- This receptor (now called a secretory component) is retained as protection from proteolytic cleavage of IgA.
- The secondary immune response is more rapid, is larger, has higher affinity, and requires less antigenic stimulation than the primary response.
- The immunoglobulins of the secondary immune response are IgG, IgA, and IgE.

Review Questions

1. A patient is suffering from lymphadenopathy and splenomegaly. He has greatly increased numbers of lymphocytes, reduced numbers of platelets, and autoimmune anemia. When his peripheral blood leukocytes are exposed to T-cell mitogens, they proliferate wildly, even for weeks after the mitogenic stimulus is removed. Which of the following is most likely to be the genetic defect?

   (A) Absence of complement
   (B) Absence of Fas
   (C) Absence of interferon-γ
   (D) Absence of perforin
   (E) Absence of TNF

2. Up until the 1970s, tonsillectomies were routinely performed on children with swollen tonsils. This procedure has lost its widespread appeal as we have learned the important role of mucosal-associated lymphoid tissue (MALT) in the protective immune response. What is the major immunoglobulin produced by the MALT?

   (A) A dimeric immunoglobulin with secretory component
   (B) A monomeric immunoglobulin that crosses the placenta
   (C) A monomeric immunoglobulin bound by mast cells
   (D) A monomeric immunoglobulin that opsonizes
   (E) A pentameric immunoglobulin that activates complement

3. In a lifetime, a person may receive a dozen or more tetanus toxoid inoculations. When boosters are administered at 10-year intervals, which of the following would be true of the B lymphocytes that respond?

   (A) Their receptors would have high avidity
   (B) They would be large and highly metabolic
   (C) They would have low levels of adhesion molecules
   (D) They would have surface IgG, IgA, or IgE
   (E) They would have surface IgM

4. A 64-year-old man undergoes surgery to excise 18 inches of bowel with adenocarcinoma. When the tissue and draining mesenteric lymph nodes are sent for pathologist's examination, the Peyer patches are noted to be hyperplastic with IgA-secreting plasma cells, but there is no secretory IgA found in the lumen of the colon. Which of the following changes in the bowel epithelium could explain this finding?

   (A) Failure of isotype switching
   (B) Failure of variable domain gene-segment rearrangement
   (C) Loss of J chain synthesis
   (D) Loss of the polyimmunoglobulin receptor
   (E) Loss of TH2 cells

5. An immunologic laboratory is studying the migration patterns of different lymphocyte subpopulations. One population of small, nondividing lymphocytes, which are CD3+ and CD4+ and express low levels of IL-2 receptors but high levels of LFA-1, are labeled with a radioactive marker and traced as they migrate through the body. What type of cell has been labeled in this case?

   (A) A blast cell
   (B) A memory T cell
   (C) An activated T cell
   (D) A naive T cell
   (E) An effector T cell

Answers and Explanations

1. **The correct answer is B.** This patient has Canale-Smith syndrome, a condition in which a mutant Fas protein is produced. This mutant protein competes with the normal proteins essential for the Fas-mediated death pathway. For this reason, these patients develop a progressively increasing and ultimately unsustainable number of lymphocytes of specific clones and the inability to mount any new protective response.

   Absence of complement (**choice A**) is not a condition that would result in a failure of homeostasis in immune cells. Complement is activated by invasion of foreign pathogens or by the complexes of antigen and antibody. Neither of these is believed to play a role in the dampening of specific immune responses when they are no longer necessary.

   Absence of interferon-γ (**choice C**) would tend to promote immune deviation toward the TH2 arm of the immune response and promote antibody synthesis. It would not cause uncontrolled cell proliferation or an inability to remove unnecessary cells. This cytokine is a product of TH1 cells, which then inhibits the function of TH2 cells.

   Absence of perforin (**choice D**) would inhibit the ability of cytotoxic T lymphocytes or NK cells to kill their specific targets. It is not a major mechanism involved in homeostasis of lymphocyte populations in the body.

   Absence of tumor necrosis factor (TNF) (**choice E**) would cause decreased killing of some tumor cell targets and decreased phagocytosis. Neither TNF-α nor TNF-β is believed to be involved in the homeostasis of lymphocyte populations in the body.

2. **The correct answer is A.** The mucosal-associated lymphoid tissues (MALT) are the major sites of synthesis of IgA. IgA is a dimeric molecule held together by a J chain similar to that used in IgM. As IgA is transported across the epithelial surface, it acquires the secretory component, which functions both in transepithelial transport and protection from proteolytic cleavage.

   A monomeric immunoglobulin that crosses the placenta (**choice B**) describes IgG. IgG is the major immunoglobulin of the blood and is produced in lymph nodes and spleen, but less commonly in the MALT.

   A monomeric immunoglobulin bound by mast cells (**choice C**) describes IgE. IgE is the immunoglobulin that causes immediate hypersensitivity by virtue of its attraction to the Fc receptors of mast cells. It is not the major immunoglobulin produced in the MALT, although it may be produced there.

   A monomeric immunoglobulin that opsonizes (**choice D**) describes IgG. IgG is the major immunoglobulin of the blood and is produced in lymph nodes and spleen, but less commonly in the MALT.

   A pentameric immunoglobulin that activates complement (**choice E**) describes IgM. IgM is the major immunoglobulin of the primary immune response and is produced in lymph nodes and spleen, but less commonly in the MALT.

3. **The correct answer is D.** The protective response to the tetanus toxoid depends on production of antibodies that prevent the binding of the toxin. After repeated immunizations, the population of memory B cells is stimulated, which is the goal of such prophylaxis. Memory B cells may have IgG, IgA, or occasionally IgE on their surfaces serving as antigen receptors.

   That their receptors would have high avidity (**choice A**) is not true because avidity decreases with repeated booster inoculations. This is because IgM, which is the immunoglobulin of the primary immune response and is the receptor on mature naive B lymphocytes, is replaced in secondary and subsequent responses by isotype switching to other isotypes such as IgG or IgA or IgE. All of these molecules have less avidity than IgM

because they have fewer combining sites than IgM. The secondary and subsequent responses should have increased affinity (goodness-of-fit of idiotype for epitope), but decreased avidity.

That they would be large and highly metabolic (**choice B**) is not true because memory lymphocytes are usually small and in a resting phase of the cell cycle. Activated lymphocytes are large and highly metabolic.

That they would have low levels of adhesion molecules (**choice C**) is not true because memory lymphocytes express high levels of adhesion molecules. This allows them to migrate to areas of active inflammation where they can have maximum benefit in protection of the host.

That they would have surface IgM (**choice E**) is not true because this would describe mature, naive B lymphocytes that have not met their antigen before. As soon as the primary response begins, isotype switching to other classes of immunoglobulin is directed by TH cells.

4. **The correct answer is D.** The transport of IgA dimers from the abluminal side of the mucosa to the lumen is mediated via attachment to polyimmunoglobulin receptors on mucosal cells. This allows endocytosis of IgA into the mucosal cell and secretion onto the other side. Secretory IgA be found in the lumen of the bowel retains a residue of this receptor, secretory component, which further protects it from proteolytic cleavage inside the intestine. If this receptor were lacking, transport of IgA across the mucosa would not be possible, and the IgA dimers would be trapped on the abluminal side of the mucosa.

   Failure of isotype switching (**choice A**) is not a potential cause of such a condition because isotype switching occurs in secondary lymphoid organs and not in epithelial cells. Because the IgA dimers were present, isotype switching had been successful, but transepithelial transport was not occurring.

   Failure of variable domain gene segment rearrangement (**choice B**) is not a potential cause of such a condition because variable domain gene-segment rearrangement occurs in the primary lymphoid organs and not in epithelial cells. Because immunoglobulin was being produced, these gene segment rearrangements had occurred successfully, but transepithelial transport was not occurring.

   Loss of J chain synthesis (**choice C**) would result in the inability of an individual to join dimers of IgA and pentamers of IgM. Because the question states that the individual was making IgA dimers, J chain is clearly being made successfully by the B cell.

   Loss of TH2 cells (**choice E**) would cause the patient to be unable to switch isotypes. These persons could make only IgM, and this patient clearly has successfully produced IgA.

5. **The correct answer is B.** This is a population of memory T lymphocytes (small, nonmitotic cells rich in adhesion molecules).

   A blast cell (**choice A**) is a cell undergoing blastogenesis (rapid proliferation or cloning). These cells would be large and highly mitotic and possess high levels of adhesion molecules.

   An activated T cell (**choice C**) would be large and highly mitotic and possess high levels of adhesion molecules.

   Naive T cells (**choice D**) would be small with scant cytoplasm. They would be nonmitotic and express low levels of adhesion molecules.

   An effector T cell (**choice E**) would be large and highly mitotic and express high levels of adhesion molecules on its surface.

Vaccination and Immunotherapy

10

What the USMLE Requires You to Know

- The clinical applications of active, passive, natural, and artificial immunization
- The immunologic rationales for standard vaccination protocols
- The ontogeny of the immune response in children as it relates to vaccination, diagnosis of prenatal infection, and detection of immunodeficiency diseases
- The role of adjuvants in vaccination

Immunity to infectious organisms can be achieved by **active** or **passive immunization**. The goal of passive immunization is transient protection or alleviation of an existing condition, whereas the goal of active immunization is the elicitation of protective immunity and immunologic memory. Active and passive immunization can be achieved by both **natural** and **artificial** means.

In A Nutshell

Immunization

- Active
- Passive
- Natural
- Artificial

Table II-10-1. Types of Immunity

Type of Immunity	Acquired Through	Examples
Natural	Passive means	Placental IgG transport, colostrum
Natural	Active means	Recovery from infection
Artificial	Passive means	Horse antivenin against black widow spider bite, snake bite Horse antitoxin against botulism, diphtheria Pooled human immune globulin versus hepatitis A and B, measles, rabies, or tetanus "Humanized" monoclonal antibodies versus RSV*
Artificial	Active means	Hepatitis B component vaccine Diphtheria, tetanus, pertussis toxoid vaccine *Haemophilus* capsular vaccine Polio live or inactivated vaccine Measles, mumps, rubella attenuated vaccine Varicella attenuated vaccine

*Monoclonal antibodies prepared in mice but spliced to the constant regions of human IgG

In A Nutshell

Passive immunotherapy can cause:

- IgE production
- Type III hypersensitivity
- Anti-allotype antibodies

Passive immunotherapy may be associated with several risks:

- Introduction of antibodies from other species can generate IgE antibodies, which may cause systemic anaphylaxis (*see* Chapter 13).
- Introduction of antibodies from other species can generate IgG or IgM anti-isotype antibodies, which form complement-activating immune complexes, which can lead to type III hypersensitivity reactions (*see* Chapter 13).
- Introduction of antibodies from humans can elicit responses against minor immunoglobulin polymorphisms or **allotypes.**

Persons with selective IgA deficiency (1:700 in population, *see* Chapter 11) are at risk to develop reactions against infused IgA (a molecule they have not seen before).

Active immunization (**vaccination**) has played an important role in the reduction of morbidity and mortality from various infectious diseases, especially among children, and is currently recommended on the following schedule:

Recommended Childhood and Adolescent Immunization Schedule -- United States, 2004

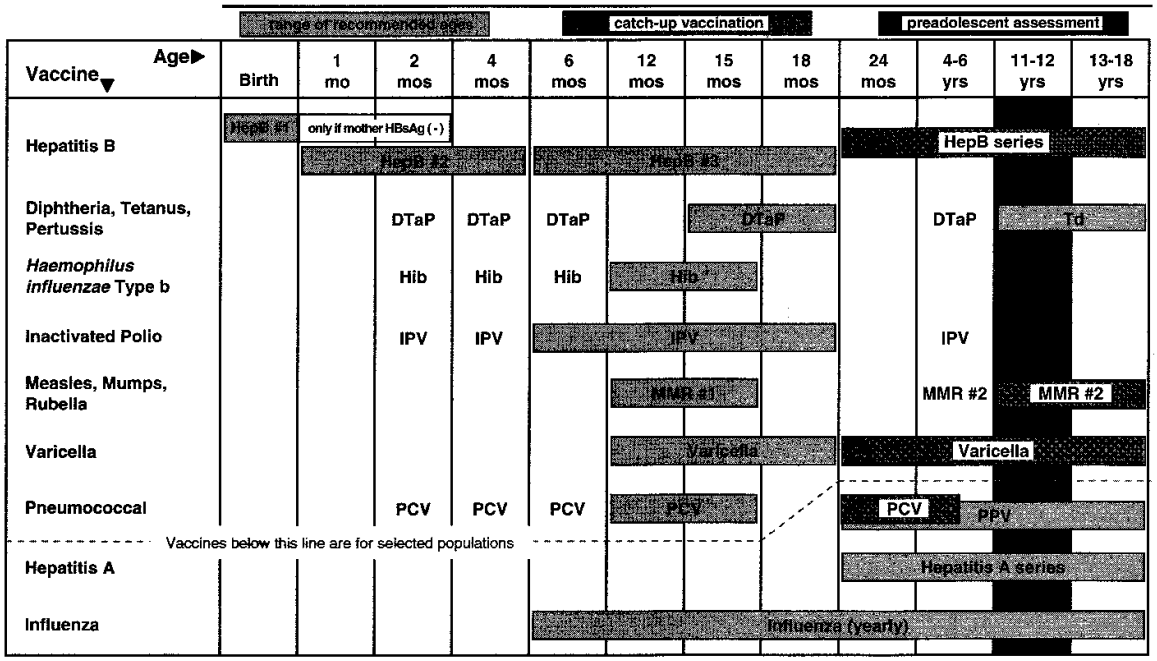


Figure II-10-1

Note

Live viral vaccines are never safe for immunocompromised patients.

Many of these vaccinations require multiple booster inoculations in children to achieve maximal protection. Live attenuated virus vaccines are only given after 12 months of age because residual maternal antibodies would inhibit replication and the vaccine would fail. In cases where children are at exceptionally high risk for exposure to a pathogen, this rule is sometimes broken, but administration of vaccines earlier than 6 to 9 months of age is almost always associated with the need for repeated booster inoculations.

Live viral vaccines should never be given to immunocompromised patients because even attenuated live viruses could cause significant pathology in these individuals.

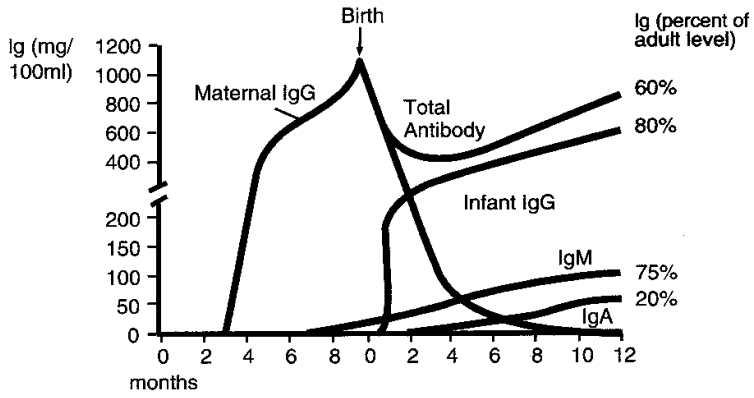


Figure II-10-2. Immunoglobulins in the Serum of the Fetus and Newborn Child

One of the key factors for consideration in the design of vaccines is the arm of the immune response that needs to be stimulated to produce a protective response. Thus, although humoral responses are usually adequate to neutralize bacterial toxins or block virus binding, if TH cells are not elicited during this process, the immune response generated will display no immunologic memory. For this reason, the vaccine for *Haemophilus influenzae* type B in children was engineered in such a way that the capsular polysaccharide of the organism was covalently coupled to a protein carrier (either *Neisseria meningitidis* outer-membrane proteins or the diphtheria toxoid), so that T-cell recognition of the protein carrier would serve to provide the activated TH cells necessary for the generation of IgA and IgG antibodies and immunologic memory. Two other polysaccharide capsular vaccines, against *Streptococcus pneumoniae* and *Neisseria meningitidis*, are not administered with a protein component, but may elicit protective immunity by stimulating previously existing reactive cells formed in response to previous infection or normal flora cross-reactivities.

As a general rule, viral vaccines that are living strains elicit both cell-mediated and humoral immunity, whereas killed viral vaccines elicit predominantly an antibody response.

Recombinant Antigen Vaccines

The hepatitis B vaccine is produced by recombinant DNA technology. In this way, the gene coding for the HBsAg is inserted into yeast cells, which then release this molecule into the culture medium. The molecule is then purified and used as the immunogen in the vaccine.

Recombinant Vector Vaccines

Attenuated viral or bacterial organisms can be used as vectors for the introduction of antigens from harmful pathogens. Vaccinia virus has been used as a vector in this way by inserting gene products from several sources, which will then be produced by the modified vector when inoculated into a host. If the foreign gene product is a viral envelope protein, it is inserted into the membrane of the infected host cell and induces the development of both cell-mediated and humoral immunity. This strategy is being used in clinical trials of HIV antigens introduced into canarypox vectors.

Clinical Correlate

- Persistence of maternal Ab affects vaccinations.
- Infant has 20% of adult IgA at 12 months, so colostrum is important.
- IgM is the only isotype useful in diagnosing infections in neonate.
- Normal infants have few infections during first few months because of maternal IgG.
- Children with immune deficiencies don't become ill until maternal IgG is low.

In A Nutshell

- HIB vaccine is a T-cell–dependent vaccine.
- Living viral vaccines elicit CMI and HMI.
- Killed viral vaccines elicit antibodies.

In A Nutshell

Hepatitis B vaccine is produced by recombinant DNA.

In A Nutshell

Experimental Vaccine Strategies

- Recombinant vector
- DNA vaccines

DNA Vaccines

In this vaccination strategy, plasmid DNA encoding antigenic proteins is injected directly into the muscle of the recipient. The DNA is taken up by muscle cells and dendritic cells in the area, and the protein is expressed in its natural form, without denaturation or modification. These vaccines elicit both humoral and cell-mediated responses, and the prolonged expression of the molecule generates significant immunologic memory.

In A Nutshell

Adjuvants increase immunogenicity nonspecifically.

Adjuvants

Adjuvants are substances that increase the immunogenicity of an antigen when administered with it. They may exert one or more of the following effects:

- Prolonging antigen persistence (aluminum potassium sulfate)
- Enhancing costimulatory signals (muramyl dipeptide)
- Inducing granuloma formation (alum)
- Inducing nonspecific lymphocyte proliferation (lipopolysaccharide and synthetic polyribonucleotides)

Chapter Summary

- Active immunization occurs when an individual is exposed (naturally or artificially) to a pathogen.
- Passive immunization occurs when an individual receives preformed immune products (antibodies, cells) against a pathogen (naturally or artificially).
- Passive immunotherapy is useful in postexposure prophylaxis but runs the risk of eliciting adverse immune responses (hypersensitivity).
- Childhood vaccination protocols must take into account risk of exposure, presence of maternal antibodies, and the type of protective immune response needed.
- Only killed or component vaccines are safe for use in immunocompromised patients.
- Live viral vaccines elicit both cellular and humoral responses, whereas killed viral vaccines elicit primarily antibody responses.
- The hepatitis B vaccine is a component vaccine produced by recombinant DNA technology.
- Recombinant vector vaccines and DNA vaccines are in experimental preparation.
- Adjuvants increase immunogenicity nonspecifically.

Review Questions

1. A 10-year-old child was bitten by a stray dog. The child is started on a course of inoculations of pooled human antirabies immunoglobulin. What is repeated inoculation of this antirabies immunoglobulin preparation likely to induce?

   (A) Anti-allotype antibodies
   (B) Anti-epitope antibodies
   (C) Anti-idiotype antibodies
   (D) Anti-isotype antibodies
   (E) Anti-rabies antibodies

2. All residents of a Chicago nursing home are inoculated intramuscularly with an H3N2 influenza A preparation. The goal of this protocol is to stimulate which of the following types of immunity?

   (A) Adaptive
   (B) Artificial active
   (C) Artificial passive
   (D) Natural active
   (E) Natural passive

3. A city sanitation worker is struck by a car and his leg is crushed against his sanitation truck. The extreme trauma to the leg necessitates amputation above the knee. Although the patient's health records reflect a tetanus booster 6 years ago, the man is revaccinated; human, pooled antitetanus immunoglobulin is injected around the macerated tissue. Administration of immunoglobulin is an example of which of the following forms of immunization?

   (A) Adaptive
   (B) Artificial active
   (C) Artificial passive
   (D) Natural active
   (E) Natural passive

4. A 28-year-old man was brought into court for nonpayment of child support. A 20-year-old woman insists that he is the father of her child. The court suggests before hearing the paternity case that various genetic tests be performed on the man, woman, and child. One of the sets of tests was for genetic immunoglobulin identification. Which immunoglobulin marker would be useful in this case?

   (A) Allotype
   (B) Idiotype
   (C) IgA2
   (D) IgM
   (E) Isotype

5. In 1988 a new childhood vaccine was developed to protect against epidemic meningitis by mixing *Haemophilus influenzae* type B capsular polysaccharide with whole, killed *Bordetella pertussis* bacteria. The function of the whole, killed bacteria in this vaccine is as a(n)

   (A) carrier
   (B) hapten
   (A) mitogen
   (D) adjuvant
   (E) immunogen

Answers and Explanations

1. **The correct answer is A.** Because rabies antitoxin is a pooled, human immunoglobulin product, repeated inoculation will cause a patient to produce anti-allotype antibodies. Allotypes are minor amino-acid sequence variations in the constant domains of heavy and light immunoglobulin chains. Their expression is genetically determined, and repeated exposure to molecules of foreign allotype can cause the production of antibodies, which recognize these sequence variations.

   Anti-epitope antibodies (**choice B**) would be produced by repeated inoculation of an immunogen. The epitope of the antigen has a three-dimensional complementarity with the idiotype of the antibody molecule. In this case, anti-epitope antibodies would be generated by rabies vaccination, but the question asks what the result of repeated exposure to immunoglobulins would be.

   Anti-idiotype antibodies (**choice C**) would be generated in a human if a monoclonal antibody preparation were repeatedly inoculated into another human. The idiotype of an antibody is the three-dimensional shape of its antigen-combining site. It is unique to the antibodies produced by a clone of cells. Because the material mentioned in this case is a pooled human immunoglobulin, it would contain many different idiotypes and would be unlikely to elicit any one specific anti-idiotype antibody.

   Anti-isotype antibodies (**choice D**) are usually raised across species barriers. For example, to produce anti-human IgG, IgG pooled from many humans is repeatedly injected into rabbits, goats, or sheep. These animals will recognize the human determinants in the constant domains of the heavy and light chains (the isotypes) and will produce antibodies that specifically recognize those determinants.

   Anti-rabies antibodies (**choice E**) are generated during vaccination. When the killed virus is administered, the patient makes an active, artificial response to the immunogen and produces immunoglobulins, which will protect against virus attachment. In this case, anti-rabies antibodies were inoculated, so there is no possibility that more of the same will be generated.

2. **The correct answer is B.** In this case, high-risk individuals are vaccinated with the serotype of influenza virus that is predicted to be most common in this flu season. This elicits an active immunologic response in the patient and is artificial by definition because it is being administered in a medical setting. This sort of immunization causes the development of memory in the patient that will protect for the whole season, but it requires approximately two weeks for development of protection.

   Adaptive (**choice A**) immunity describes all immune responses that have specificity and memory. These immune responses are produced by specific B and T lymphocytes. Although adaptive immunity will be elicited in these patients, this is not the best answer because it is imprecise.

   Artificial passive (**choice C**) immunity is achieved when preformed immunologic products (immune cells or antibodies) are given to a patient. These procedures provide passive protection that is rapid but lacks immunologic memory. Because it is administered in a medical setting, it is, by definition, artificial.

   Natural active (**choice D**) immunity would result following recovery from an infection.

   Natural passive (**choice E**) immunity is acquired across the placenta and in the colostrum and breast milk, from mother to child. The child receives preformed antibodies (IgG across the placenta and IgA in milk) that protect the child until a natural active immune response can be mounted.

3. **The correct answer is C.** In this case, an attempt at postexposure prophylaxis against tetanus is made by inoculating antitetanus immunoglobulin into the patient. When preformed immunologic products (immune cells or antibodies) are given to a patient, the procedure provides passive protection that is rapid but lacks immunologic memory. Because it is being administered in a medical setting, it is by definition artificial.

   Adaptive (**choice A**) immunity describes all immune responses that have specificity and memory. These immune responses are produced by specific B and T lymphocytes. Because this patient is being given a product of the adaptive immune response (antibodies), there will be no elicitation of an adaptive immune response in this individual.

   Artificial active (**choice B**) immunity is produced during the process of vaccination. The patient is exposed to a modified pathogen or product. As a result, an active immune response to that inoculation is made. This sort of immunization causes the development of memory in the patient.

   Natural active (**choice D**) immunity would result after a recovery from an infection.

   Natural passive (**choice E**) immunity is acquired across the placenta and in the colostrum and breast milk, from mother to child. The child receives preformed antibodies (IgG across the placenta and IgA in milk), which serve to protect the child until a natural active immune response can be mounted.

4. **The correct answer is A.** Allotypes are minor amino-acid sequence variations in the constant domains of heavy and light immunoglobulin chains. Their expression is genetically determined, and variations can be used as evidence in favor of paternity in some cases. Allotypic markers are most frequently used in studies of population genetics, as certain ethnic groups are likely to have similar allotypic markers on their immunoglobulins. Allotypic markers do not affect the biologic function of the immunoglobulin molecule.

   The term "idiotype" (**choice B**) describes the three-dimensional shape of the antigen-combining site of an antibody or T-cell receptor molecule. Because each human is capable of producing many millions of different idiotypic sequences, these would not be useful in paternity cases.

   IgA2 (**choice C**) is an isotype of immunoglobulin. Because all normal human beings produce some amount of this immunoglobulin, it would not be useful in paternity cases.

   IgM (**choice D**) is an isotype of immunoglobulin. Because all normal human beings produce some amount of this immunoglobulin, it would not be useful in paternity cases.

   An isotype (**choice E**) is the heavy- or light-chain constant domain of an immunoglobulin. Thus, there are five heavy-chain isotypes (A, E, G, M, and D) and two light-chain isotypes (κ and λ). Because all human beings produce heavy- and light-chain isotypes, this would not be useful in paternity testing.

5. **The correct answer is D.** Although this vaccine is no longer in use because of the possible side effects of *Bordetella pertussis* inoculation, in this case the whole, killed bacteria served as an adjuvant. They increased local inflammation, thus calling inflammatory cells to the site and prolonging exposure to the immunogen, the capsular polysaccharide of *Haemophilus*.

   A carrier (**choice A**) is not correct because a carrier is a protein covalently coupled to a hapten to elicit a response. There is no mention in the question stem here that the polysaccharide is chemically coupled to the bacteria; it is stated that they are only mixed together.

   A hapten (**choice B**) is not correct because a hapten is a single antigenic epitope, and a whole, killed bacterium such as *Bordetella* has many epitopes.

A mitogen (**choice C**) is not correct because mitogens are substances that cause the polyclonal activation of immune cells. The mitogens most commonly used in clinical laboratory medicine are lipopolysaccharide, concanavalin A, and pokeweed mitogen.

An immunogen (**choice E**) is not correct because the immunogen in a vaccine is the substance to which the immune response is being made. Because the object of the HIB vaccine is to immunize against *Haemophilus influenzae, Bordetella pertussis* bacteria cannot be the immunogen.

Immunodeficiency Diseases

11

What the USMLE Requires You to Know

The molecular defects, signs, and symptoms associated with defects of phagocytic cells, complement, and B and T cells.

If individuals experience defects in the functioning of any of the components of the immune system, clinical manifestations are common. These **immunodeficiency diseases** are favorite topics of USMLE vignettes and are reviewed here in their totality, although many have been discussed previously in the Clinical Correlates spread throughout the chapters.

Defects of Phagocytic Cells

Table II-11-1. Defects of Phagocytic Cells

Disease	Molecular Defect(s)	Symptoms
Chronic granulomatous disease (CGD)	Deficiency of NADPH oxidase (any one of four component proteins); failure to generate superoxide anion, other O_2 radicals	Recurrent infections with catalase-positive bacteria and fungi
Chediak-Higashi syndrome	Granule structural defect	Recurrent infection with bacteria: chemotactic and degranulation defects; absent NK activity, partial albinism
Glucose-6-phosphate dehydrogenase (G6PD) deficiency	Deficiency of essential enzyme in hexose monophosphate shunt	Same as CGD, with associated anemia
Myeloperoxidase deficiency	Granule enzyme deficiency	Mild or none
Leukocyte adhesion deficiency	Absence of CD18—common β chain of the leukocyte integrins	Recurrent and chronic infections, fail to form pus, and do not reject umbilical cord stump

Defects of Humoral Immunity

Table II-11-2. Defects of Humoral Immunity

Disease	Molecular Defect	Symptoms/Signs	Treatment
Bruton X-linked hypogammaglobulinemia	Deficiency of a tyrosine kinase blocks B-cell maturation	Low immunoglobulin of all classes, no circulating B cells, pre-B cells in bone marrow in normal numbers, normal cell-mediated immunity	Monthly gammaglobulin replacement, antibiotics for infection
Transient hypogammaglobulinemia of infancy	Delayed onset of normal IgG synthesis	Detected in 5th to 6th month of life, resolves by 16–30 months; susceptibility to pyogenic bacteria	Antibiotics and in severe cases, gammaglobulin replacement
Common variable hypogammaglobulinemia	Unknown	Onsets in late teens, early twenties; B cells present in peripheral blood, immunoglobulin levels decrease with time; increased autoimmunity	Antibiotics
Selective IgA deficiency	Deficiency of IgA (most common)	Repeated sinopulmonary and gastrointestinal infections	Antibiotics, not immunoglobulins
X-linked hyper-IgM syndrome	Deficiency of CD40L on activated T cells	High serum titers of IgM without other isotypes, normal B- and T-cell numbers, susceptibility to extracellular bacteria and opportunists	Antibiotics and gammaglobulins

Deficiencies of Complement or Its Regulation

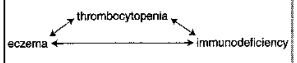
Table II-11-3. Deficiencies of Complement or Its Regulation

Deficiencies in Complement Components	Deficiency	Signs/Diagnosis
Classic pathway	C1q, C1r, C1s, C4, C2	Marked increase in immune complex diseases, increased infections with pyogenic bacteria
Alternative pathway	Factor B, properdin	Increased *Neisseria* infections
Both pathways	C3	Recurrent bacterial infections, immune complex disease
	C5, C6, C7, or C8	Recurrent meningococcal and gonococcal infections
Deficiencies in complement regulatory proteins	C1-INH (hereditary angioedema)	Overuse of C1, C4, or C2 Edema at mucosal surfaces
	Decay-activating factor or homologous restriction factor	Paroxysmal nocturnal hemoglobinuria

Defects of T Lymphocytes and Severe Combined Immunodeficiencies

Although patients with defects in B lymphocytes can deal with many pathogens adequately, defects in T lymphocytes are observed globally throughout the immune system. Because of the central role of T cells in activation, proliferation, differentiation, and modulation of virtually all naturally occurring immune responses, abnormalities in these cell lines send shock waves throughout the system. It is often a Herculean clinical effort to dissect the cause-and-effect relationships in such inherited diseases, and their diagnosis is often one of trial-and-error, which takes years to unravel. Although in some cases both B- and T-lymphocyte defects may occur, the initial manifestation of these diseases is almost always infection with agents such as **fungi and viruses** that are normally destroyed by T-cell–mediated immunity. The B-cell defect, if any, is usually not detected for the first few months of life because of the passive transfer of immunoglobulins from the mother through the placenta or colostrum. The immune system is so compromised that even attenuated vaccine preparations can cause infection and disease.

Table II-11-4. B- and T-Cell Deficiencies

Category	Disease	Defect	Clinical Manifestations
Selective T-cell deficiency	DiGeorge Syndrome	Failure of formation of 3rd and 4th pharyngeal pouches, thymic aplasia	Facial abnormalities, hypoparathyroidism, cardiac malformations, depression of T-cell numbers, and absence of T-cell responses
	MHC class I deficiency	Failure of TAP 1 molecules to transport peptides to endoplasmic reticulum	CD8+ T cells deficient, CD4+ T cells normal, recurring viral infections, normal DTH, normal Ab production
Combined partial B- and T-cell deficiency	Wiskott-Aldrich Syndrome	Defect in cytoskeletal glycoprotein, X-linked	Defective responses to bacterial polysaccharides and depressed IgM, gradual loss of humoral and cellular responses, thrombocytopenia, and eczema 
	Ataxia telangiectasia	Defect in kinase involved in the cell cycle	Ataxia (gait abnormalities), telangiectasia (capillary distortions in the eye), deficiency of IgA and IgE production
Complete functional B- and T-cell deficiency	Severe combined immunodeficiency (SCID)	Defects in common γ chain of IL-2 receptor (present in receptors for IL-4, -7, -9, -15), X-linked	Chronic diarrhea; skin, mouth, and throat lesions; opportunistic (fungal) infections; low levels of circulating lymphocytes; cells unresponsive to mitogens
		Adenosine deaminase deficiency (results in toxic metabolic products in cells)	See above
		Defect in signal transduction from T-cell IL-2 receptors	See above
		Bare lymphocyte syndrome/ MHC class II deficiency	T cells present and responsive to nonspecific mitogens, no GVHD, deficient in CD4+ T cells, hypogammaglobulinemia

Chapter Summary

- B-cell, phagocyte, and complement defects predispose to infections with extracellular pathogens.
- T-cell defects predispose to infections with intracellular pathogens.
- Severe combined immunodeficiencies tend to manifest first as T-cell defects.

Review Questions

1. A newborn is evaluated for immunologic function. He has a distortion of the shape of his mouth, low-set and malformed ears, and widely spaced eyes. Radiographically, there is evidence of cardiac malformation and absence of a thymic shadow. Which of the following parameters would be normal in this child?

   (A) Antibody-dependent cell-mediated cytotoxicity of parasite targets
   (B) Cellularity of splenic periarteriolar lymphoid sheaths
   (C) Cytotoxic killing of virus-infected targets
   (D) Generation of oxygen metabolites in phagocytic cells
   (E) Proliferative response to concanavalin A

2. A 14-month-old male infant is referred to a specialist for diagnosis of a potential immunologic deficiency. For the past 4 months, the child has suffered repeated episodes of bacterial infections and attempts to induce immunity using the pneumococcal vaccine have failed. Studies of peripheral blood indicate an absence of cells responsive to pokeweed mitogen. Bone marrow aspirates are remarkable for hypercellularity of pro-B cells. What is the most likely diagnosis?

   (A) Bruton agammaglobulinemia
   (B) Common variable hypogammaglobulinemia
   (C) DiGeorge syndrome
   (D) Selective immunoglobulin deficiency
   (E) Wiskott-Aldrich syndrome

3. A 31-year-old man is treated for a fourth episode of disseminated *Neisseria gonorrhoeae* infection in the last 5 years. He had no previous history of unusual or recurrent infections. If he has an immunologic defect, which of the following is most likely?

   (A) Common variable immunodeficiency
   (B) C8 deficiency
   (C) DiGeorge syndrome
   (D) Selective IgA deficiency
   (E) Severe combined immunodeficiency

4. A patient has been hospitalized three times for painful abdominal edema and is complaining now of swollen lips. What will laboratory findings in this patient most likely include?

   (A) Abnormal superoxide anion production by neutrophils
   (B) Abnormal T-cell function
   (C) Abnormal T-cell numbers
   (D) Defective neutrophil chemotaxis
   (E) Reduced C4 levels

5. A 4-year-old girl presents with a severe *Staphylococcus aureus* abscess. Her history is significant for a previous infection with *Serratia marcescens*. If she has an enzyme deficiency, which of the following is most likely?

   (A) Adenosine deaminase
   (B) C1 inhibitor
   (C) Myeloperoxidase
   (D) NADPH oxidase
   (E) Superoxide dismutase

6. A new pediatrician has just opened his office next to the hospital, and one of his first patients is a small, 4-year-old boy. His mother brings him into the office because he has several boil-like lesions on his arm. His mother told the physician that the boy has had these lesions on several different occasions. His other physician had prescribed antibiotics, and the lesions resolved. His records indicate he has had all of his immunizations. The pediatrician orders several different laboratory tests, and the following results are reported:

Immunoglobulin levels	normal
B-cell and T-cell counts	normal
Complement levels	normal
Calcium and parathyroid hormone levels	normal
Nitroblue tetrazolium test	negative

   The mother told the physician that she was not aware of any eczema or bleeding problems. Which disease is indicated by these findings?

   (A) Bruton X-linked agammaglobulinemia
   (B) Chronic granulomatous disease
   (C) DiGeorge syndrome
   (D) Severe combined immunodeficiency disease
   (E) Wiskott-Aldrich syndrome

7. An acutely ill, 2-year-old boy is hospitalized with *Staphylococcus aureus* pneumonia, which is treated appropriately. The patient's history indicates similar bouts of bacterial infections in the past. He had recovered uneventfully from measles 6 months ago. Physical examination discloses scant tonsillar tissue and no palpable lymphadenopathy. Immunoelectrophoresis reveals subnormal levels of gammaglobulins. The nitroblue tetrazolium and chemiluminescence assays indicate normal phagocytic killing. Which of the following disorders is most likely responsible for this child's condition?

   (A) Adenosine deaminase deficiency
   (B) Defect of the *Btk* gene
   (C) Defect of the *SAP* gene
   (D) Defect of the *WAS* gene
   (E) ICAM-1 deficiency

8. A 2-year-old boy suffering from repeated painful bouts of inflammation of mucosal surfaces, especially affecting the lips, is brought to the pediatrician's office. The mother remembers similar symptoms in previous generations of her family and fears a heritable tendency toward food allergy. What laboratory finding would best support the physician's suspicion?

   (A) Depressed C3
   (B) Depressed C4
   (C) Depressed C5
   (D) Elevated C1
   (E) Elevated C1, C4, and C2

Answers and Explanations

1. **The correct answer is D.** This is a case of DiGeorge syndrome, which is a congenital failure in the formation of the third and fourth pharyngeal pouches. As a result, individuals with this defect have aplastic thymus and parathyroids and facial, esophageal, and cardiac malformations. Immunologically, the absence of the thymus will ultimately have global effects on the development of all T-cell–mediated immune responses. At birth, the child will have IgG antibodies that have been transplacentally transferred from the mother, but by 12 months or so after birth, these will be gone and IgM will be the only isotype of immunoglobulin present. Phagocytic killing will be normal until that point, although after all the maternal IgG is gone, opsonization of bacteria will no longer be possible.

   Antibody-dependent cell-mediated cytotoxicity of parasite targets (**choice A**) will be depressed in this child because eosinophil-mediated ADCC requires IgE antibodies, and these cannot be produced without T-cell help.

   Cellularity of splenic periarteriolar lymphoid sheaths (**choice B**) will be decreased in this child because these are T-cell–dependent areas of the spleen.

   Cytotoxic killing of virus-infected targets (**choice C**) will be depressed in this child because cytotoxic T cells will be absent, and only NK cells will be available for antiviral protection.

   The proliferative response to concanavalin A (**choice E**) will be depressed in this child because concanavalin A is a T-cell mitogen. If there are no T cells, there will be no proliferation in response to this mitogen.

2. **The correct answer is A.** This is a case of X-linked agammaglobulinemia, or Bruton agammaglobulinemia. It is caused by a mutation in a thymidine kinase gene, which is important in B-cell maturation. The bone marrow becomes hypercellular with cells that cannot progress from the pro-B to the pre-B stage, while the peripheral blood lacks mature B lymphocytes. There will be no proliferative response to B-cell mitogens (pokeweed mitogen), and CD19+ cells will be absent from the blood. Persons with this condition are unable to mount a normal antibody response; therefore, symptoms appear after the disappearance of maternal antibodies. Susceptibility to extracellular, encapsulated pathogens is profound.

   Common variable hypogammaglobulinemia (**choice B**) is a condition that usually appears in the late teens or early twenties. It is believed to be an autoimmune disease and is associated with the disappearance of immunoglobulin isotypes over time.

   DiGeorge syndrome (**choice C**), or congenital thymic aplasia, is a condition in which there is failure of formation of the third and fourth pharyngeal pouches. These infants have facial abnormalities, failure of formation of the parathyroids, and cardiac defects, as well as absence of T-lymphocyte development.

   Selective immunoglobulin deficiency (**choice D**) would not be manifested by a failure of B-cell development in the bone marrow. Selective IgA deficiency is most common of these and would manifest as increased susceptibility to mucosal-surface pathogens.

   Wiskott-Aldrich syndrome (**choice E**) is a complex immune deficiency with a triad of symptoms: eczema, thrombocytopenia, and immunodeficiency. It is inherited in an X-linked recessive fashion. These patients are prone to development of malignant lymphomas and have inability to respond to polysaccharide antigens.

3. **The correct answer is B.** Unusual frequency or severity of *Neisseria* infections should always lead to a suspicion of a terminal complement component deficiency (C5, C6, C7, or C8). *Neisseria* seem to be highly susceptible to complement-mediated lysis, so any failure of production of the membrane attack complex predisposes the patient to recurrent bacteremias with these organisms.

Common variable immunodeficiency (**choice A**) is a condition that usually appears in the late teens or early twenties. It is believed to be an autoimmune disease and is associated with the disappearance of immunoglobulin isotypes over time.

DiGeorge syndrome (**choice C**) is a condition in which there is failure of formation of the third and fourth pharyngeal pouches. Diagnosed in infancy, these individuals have facial abnormalities, failure of formation of the parathyroids, and cardiac defects, as well as an absence of T-lymphocyte development. This condition predisposes to early viral and fungal infections.

Selective IgA deficiency (**choice D**) would be expected to result in respiratory and gastrointestinal tract infections, autoimmune disease, and allergies.

Severe combined immunodeficiency (**choice E**) typically presents with early susceptibility to viral and fungal agents. It is most frequently diagnosed in infancy, after the disappearance of maternally derived IgG antibodies.

4. **The correct answer is E.** The description of painful abdominal edema and edema in the oral mucosa are typical of hereditary angioedema. This is a genetic deficiency of complement C1 inhibitor. When this important control protein is missing, there is excessive use of the classic complement pathway components, especially C4. This causes abnormal inflammation along the mucosal surfaces.

   Abnormal superoxide anion production by neutrophils (**choice A**) would result in predisposition to infections with extracellular pathogens.

   Abnormal T-cell function (**choice B**) would result in predisposition to infections with viral and fungal pathogens, not edema of the mucosal surfaces.

   Abnormal T-cell numbers (**choice C**) would result in predisposition to infections with viral and fungal pathogens, not edema of the mucosal surfaces.

   Defective neutrophil chemotaxis (**choice D**) would result in neutrophilia and failure to produce pus and abscesses in response to extracellular bacterial invasion.

5. **The correct answer is D.** The infections of this child with catalase-positive bacteria are characteristic of chronic granulomatous disease (CGD). While two thirds of CGD patients are male, one third has the autosomal recessive form of NADPH oxidase deficiency and can be female.

   Adenosine deaminase deficiency (**choice A**) produces a severe combined immunodeficiency. The infections seen are likely to be the result of T-cell deficiency (viral and fungal agents). In the absence of adenosine deaminase, deoxyadenosine phosphate builds up in T cells and is toxic to them.

   C1 inhibitor (**choice B**) is not an enzyme, and its absence does not predispose to infections. It is absent in the condition known as hereditary angioedema, represented by recurrent, painful bouts of mucosal edema.

   Myeloperoxidase (**choice C**) deficiency is normally without clinical symptoms. This is an enzyme that is important in intracellular killing in phagocytes because it causes formation of toxic halide radicals. However, because oxygen radicals are more important in intracellular killing, no symptoms of MPO deficiency will be observed.

   Superoxide dismutase (**choice E**) deficiency has not been described in leukocytes, and its absence would not be likely to predispose to infection.

6. **The correct answer is B.** The negative result on the nitroblue tetrazolium dye reduction test indicates a failure of oxygen radical generation inside phagocytic cells. It is a common diagnostic, along with the neutrophil oxidative index, to diagnose chronic granulomatous disease. This is a genetic defect in the production of a subunit of NADPH oxidase and is usually diagnosed when children develop recurrent infections with catalase-positive organisms.

Bruton X-linked agammaglobulinemia (**choice A**) is caused by a mutation in a thymidine kinase gene, which is important in B-cell maturation. The bone marrow becomes hypercellular with cells that cannot progress from the pro-B to the pre-B stage, while the peripheral blood lacks mature B lymphocytes. Because the child in this case has normal immunoglobulin levels, this diagnosis is not possible.

DiGeorge syndrome (**choice C**) is a condition in which there is failure of formation of the third and fourth pharyngeal pouches. Children with this defect are diagnosed in infancy and would have an absence of T lymphocytes and deficiencies of calcium and parathyroid hormone.

Severe combined immunodeficiency disease (**choice D**) usually manifests first as a T-lymphocyte defect. The child would be susceptible to viral and fungal pathogens and have depressed levels of immunoglobulins, decreased counts of both B and T lymphocytes, and normal nitroblue tetrazolium dye reduction.

Wiskott-Aldrich syndrome (**choice E**) is a complex immune deficiency with a triad of symptoms: eczema, thrombocytopenia, and immunodeficiency. Because the mother in this case is not aware of any bleeding dyscrasias or eczema, this would be an unlikely diagnosis.

7. **The correct answer is B.** This is a case of X-linked agammaglobulinemia, or Bruton agammaglobulinemia. During the early 1990s, the gene responsible for this condition was cloned. The normal counterpart of the mutant gene encodes a protein tyrosine kinase (Bruton tyrosine kinase, *Btk*), which is important in B-cell signaling. When it is absent or altered, B lymphocytes are unable to progress from the pro-B to the pre-B cell stage in the bone marrow. Thus, the bone marrow becomes hypercellular, while the peripheral blood is lacking mature B lymphocytes. Persons with this condition are unable to mount a normal antibody response; therefore, symptoms appear after the disappearance of maternal antibodies, and susceptibility to extracellular, encapsulated pathogens such as *Streptococcus pneumoniae* and *Haemophilus influenzae* is profound.

   Adenosine deaminase deficiency (**choice A**) is an example of a severe combined immunodeficiency disease (SCID). When this enzyme is absent, toxic metabolites build up in B and T lymphocytes and cause a general failure of the immune response. It would have clinical manifestations of both B- and T-lymphocyte defects, and not exclusively B lymphocytes, as described in this case history.

   A defect of the *SAP* gene (**choice C**) is believed to cause X-linked proliferative disease, in which uncontrolled T-cell proliferation follows infection with Epstein-Barr virus. *SAP* stands for SLAM-associated protein, and SLAM (signaling lymphocytic activation molecule) is a potent T-cell coactivator.

   Defect of the *WAS* gene (**choice D**) causes Wiskott-Aldrich syndrome, in which a defect in CD43 (a cytoskeletal protein) causes defects in T cells and platelets. Patients with Wiskott-Aldrich syndrome display a triad of signs: thrombocytopenia, eczema, and immunodeficiency.

   ICAM-1 deficiency (**choice E**) would cause defects of antigen recognition and activation of lymphocytes. ICAM-1 is an adhesion molecule in the immunoglobulin superfamily of genes and is bound by LFA-1 integrin.

8. **The correct answer is B.** This is a case of hereditary angioedema, caused by a deficiency in an important complement regulatory protein, C1-INH. When it is absent, the early components of the classical complement cascade are overused. It is normally diagnosed by the finding of depressed levels of complement component C4 in the blood.

   Depressed C3 (**choice A**) would not be a correlate of C1-INH deficiency. There are separate regulatory controls on abnormal complement activation that operate at the C3 level, so this condition is rarely found.

Depressed C5 (**choice C**) would not be a correlate of C1-INH deficiency. There are separate regulatory controls on abnormal complement activation that operate at the C5 level, so this condition is rarely found.

Elevated C1 (**choice D**) would not be found in this case because the condition results in the overuse of early components of the classical complement cascade. Therefore, serum levels of C1, C4, and C2 would be decreased from normal values.

Elevated C1, C4, and C2 (**choice E**) would not be found in this case because the condition results in the overuse of early components of the classical complement cascade. Therefore, serum levels of C1, C4, and C2 would be decreased from normal values.

Acquired Immunodeficiency Syndrome

12

What the USMLE Requires You to Know

- The receptors and coreceptors of HIV
- The immunologic results of systematic TH-cell eradication
- The mechanisms through which HIV evades the immune response

Of all the newly emerging pathogens of the past century, none so efficiently and inexorably eradicates the functioning of the immune system as the **human immunodeficiency virus** (HIV). This D-type retrovirus attaches to CD4 receptors on host cells (TH cells, macrophages, and microglia) and utilizes several chemokine receptors on these cells as coreceptors. Early in the infection, the virus uses the **CCR5 chemokine receptor** and is thus predominantly **macrophage-tropic**, whereas late in the infection, the virus uses the **CXCR4 chemokine receptor** and becomes **T cell-tropic.**

HIV infection ultimately results in impaired function of both adaptive and innate immune systems. The most prominent defects arise in cell-mediated immunity, but because TH cells and macrophages are infected and destroyed, all aspects of immunity are eventually affected.

In A Nutshell

- HIV infects CD4+ cells.
- CXCR4 and CCR5 chemokine receptors are coreceptors.

Table II-12-1. Mechanisms of Immune System Destruction by the Human Immunodeficiency Virus

Virus Characteristic	Immunologic Result
Multiplication in activated lymphocytes and macrophages	Reproduces virus Increases viral load
Direct cytopathic effect on lymphocytes and macrophages	Eliminates cell- and antibody-mediated immunity
Nef gene product downregulates class I MHC expression	Makes infected cells less susceptible to CTL killing
Tat gene product	Inhibits cytokine synthesis in both infected and uninfected cells
Destruction of TH cells	Eliminates immune enhancement
Immune deviation toward TH2 response	Inhibits potentially protective CMI responses and produces antibodies that can mediate ADCC, resulting in further elimination of TH cells
Antigenic drift of the gp120	Evades antibody-mediated effector mechanisms and exhausts individual's immune capacity
Heavy glycosylation of gp120	Hides potentially protective epitopes from immune recognition

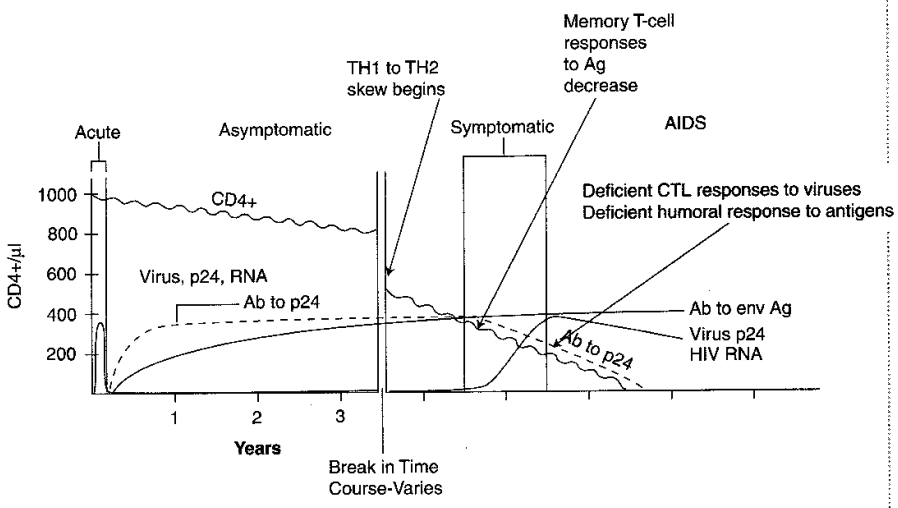


Figure II-12-1. The Course of Untreated HIV Infection and the Sequence of Immune Response Destruction

Macrophages and dendritic cells are infected with HIV, but they are resistant to its cytopathic effects. Nevertheless, they serve as reservoirs of infection, delivering virus particles into the secondary lymphoid organs and throughout the body, and their antigen-presenting function may be impaired by the infection.

HIV-infected patients exhibit both cell-mediated and humoral responses to the pathogen, but these responses are unable to eradicate the entire virus, and the infection eventually overwhelms the immune response. CD8+ cytotoxic cells are generated to kill infected CD4+ cells, and antibodies are generated to many viral antigens within 6 to 9 weeks after infection. These antibodies are used as the basis of the diagnostic tests for the infection (ELISA and Western blot, *see* Chapter 16), but there is no evidence that they play a protective role in the infection. The initial, massive CD8+ response may be responsible for the control of viremia and transition into clinical latency, but ultimately, because CD4+ cells are important in amplifying the cytotoxic response as helper numbers diminish, the amplification loop for stimulation of CD8+ cytotoxicity will be eliminated.

In A Nutshell

- Macrophages serve as reservoirs of infection.
- The immune response to the virus kills infected cells, but thereby eradicates all help.

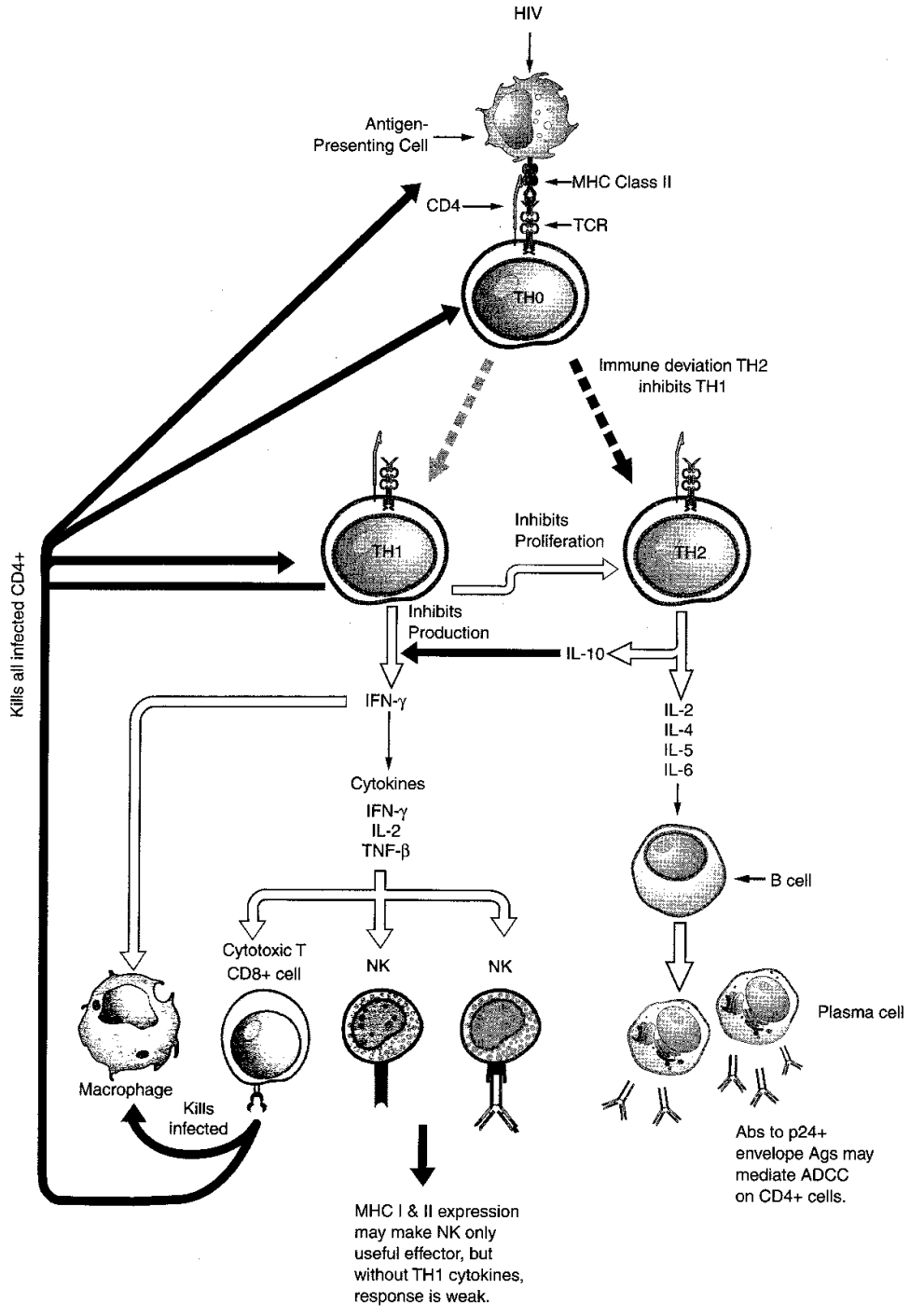


Figure II-12-2. The Global Effects of HIV on the Immune Response

Chapter Summary

- HIV infects CD4+ cells (TH cells and macrophage lineages) using CCR5 and CXCR4 chemokine coreceptors.
- HIV destroys cell-mediated and humoral immune responses by eradicating TH cells.
- HIV evades the immune response through glycosylation of surface antigens and antigenic drift.
- HIV is diagnosed using ELISA and Western blot for anti-HIV antibodies.

Review Questions

1. A 27-year-old woman who was 5 months pregnant presented for prenatal care. A routine evaluation was performed, which included testing for HIV antibody. The patient was reported to be negative for RPR but positive for HIV antibody by the EIA assay. The HIV Western blot only demonstrated the presence of an antibody to the HIV p24 antigen. How should this patient be counseled?

   (A) That she and her baby are both infected with HIV
   (B) That she is negative for HIV because the RPR is more specific
   (C) That she is positive for the HIV virus
   (D) That she should have an HIV polymerase chain reaction (PCR) test performed
   (E) That this is a confirmed false-positive HIV result

2. A neonate born to a known HIV-positive mother is evaluated for HIV status. What would be the assay of choice?

   (A) CD4 cell count
   (B) EIA for the detection of HIV antibody
   (C) Polymerase chain reaction (PCR) for the detection of HIV genome
   (D) p120 antigen assay
   (E) Western blot

3. The attachment of HIV to a TH cell is initiated by the binding of the HIV gp120 molecule to the CD4 receptor. Then gp120 undergoes a conformational change and binds to a second molecule. What is the second molecule on the surface of the CD4 lymphocyte that acts as a coreceptor and binds with the lymphotropic HIV?

   (A) The chemokine receptor CCR5 expressed on dendritic cells
   (B) The chemokine receptor CXCR4 expressed on T cells
   (C) The chemokine receptor CXCR5 expressed on T cells
   (D) The complement receptor CR2 (CD21) on B cells
   (E) The cytokine receptor CCR5 expressed on T cells

4. A male exotic dancer in San Francisco has been HIV-positive for 10 years. Because of his low income, he can only irregularly afford the antiretroviral drugs prescribed to him at the AIDS clinic. When he presents to the clinic for a checkup, his CD4 cell count is found to be 390/mm^3. Which of the following immunologic parameters is likely to be most profoundly depressed at this stage of his infection?

   (A) Cell proliferation in response to pokeweed mitogen
   (B) Delayed-type hypersensitivity to *Candida* antigens
   (C) IgA production
   (D) Intracellular killing of bacteria
   (E) Rejection of allogeneic skin grafts

5. Patients with HIV infection begin to display an immunologic deviation toward TH2 responses relatively early in the course of their disease. Which of the following cytokines would be increased in a patient with a CD4 count of 500/mm^3?

   (A) IFN-γ
   (B) IL-1
   (C) IL-2
   (D) IL-10
   (E) TNF-β

Answers and Explanations

1. **The correct answer is D.** A patient who is HIV EIA-positive must always have the result confirmed by a confirmatory assay, e.g., HIV Western blot. The HIV Western blot is considered positive when the patient demonstrates the presence of antibody to at least two of three important HIV antigens, which are gp120, gp41, and p24. If no reaction is observed, then the patient is considered negative, but any reaction that is not consistent with a positive is reported as indeterminate. This patient is considered indeterminate. The physician can wait 6 months and retest by the EIA assay, with a reflex to the Western blot assay if the EIA result is positive. If the Western blot results are identical to the previous, then the patient is reported as negative or the patient can be tested by another confirmatory test such as the PCR assay. A negative PCR in this situation would classify this patient as negative; however, it would be wise to retest the patient in 3 to 6 months if she has risk factors.

   That she and her baby are both infected with HIV (**choice A**) is not correct. Approximately 30% of the babies from untreated and <8% from treated HIV-positive mothers will be infected.

   That she is negative for HIV because the RPR is more specific (**choice B**) is not correct because RPR is not a test for HIV but a test for syphilis.

   That she is positive for the HIV virus (**choice C**) is not correct because her Western blot was indeterminate, and a confirmatory test (e.g., Western blot or PCR) must confirm the EIA assay before the patient can be reported positive.

   That this is a confirmed false-positive HIV result (**choice E**) may eventually be proven to be correct, but with the present data one does not know if the patient has been detected in the early stages of seroconversion or simply a patient who is false positive.

2. **The correct answer is C.** The neonate could not be tested for anti-HIV antibodies because the known HIV-positive mother passively transmits anti-HIV IgG antibodies to the baby through the placenta. All babies born to HIV-positive mothers will be anti-HIV antibody-positive. Because of the sensitivity of HIV antibody testing, an HIV antibody test on a specimen from a baby born to an HIV-positive mother should not be believed until 15 months of age. The test of choice is to detect the genome of the virus by PCR.

   CD4 cell count (**choice A**) is not correct because this assay is used to measure the progression of the disease and the destruction of the target CD4+ cells. It is not a diagnostic test for the presence of the virus or antiviral antibodies.

   EIA for the detection of HIV antibody (**choice B**) is incorrect because maternal antibodies can be detected in babies who are from known HIV-positive mothers for up to 12 months after birth. The HIV EIA assay is an antibody-screening test, whereas the HIV Western blot is a confirmatory test for the presence of anti-HIV antibody.

   p120 antigen assay (**choice D**) is incorrect because there is no such test as an HIV p120 antigen assay; however, there is an HIV p24 antigen assay.

   Western blot (**choice E**) is incorrect because maternal antibodies can be detected in babies who are from known HIV-positive mothers for up to 12 months after birth. The HIV EIA assay is an antibody-screening test, whereas the HIV Western blot is a confirmatory test for the presence of anti-HIV antibody.

3. **The correct answer is B.** The HIV envelope, derived from the host-cell membrane, displays viral glycoproteins gp120 and gp41. The gp120 glycoprotein has a high affinity for CD4 and is thus important in the attachment of the virus to the cell. All cells expressing CD4 are potential targets for infection with HIV. After the gp120 of the HIV binds to the CD4, it undergoes a conformational change and must then bind to a second molecule, a coreceptor, on the surface of the target cell. The variation of the gp120 molecule determines the tropism of the HIV and thus dictates which CD4+ cell can be infected.

Lymphotropic HIV virions use the CXCR4 chemokine receptor (coreceptor) expressed on T cells and require a high density of CD4 on the surface of the cell. Macrophage-tropic HIV uses the CCR5 chemokine receptor and requires only a low level of CD4 expression on the target cell.

The CCR5 chemokine receptor expressed on dendritic cells **(choice A) is not correct.** The question is addressing the infection of TH, not dendritic, cells.

The CXCR5 chemokine receptor expressed on T cells **(choice C) is not correct.** The chemokine receptor on T cells is CXCR4, not CXCR5.

The complement receptor CR2 (CD21) on B cells **(choice D)** is not correct. CR2 (CD21) is found on B cells and is not the coreceptor on TH cells for the attachment of HIV. CR2 is the receptor for EBV.

The CCR5 cytokine receptor expressed on T cells **(choice E)** is not correct. Chemokines are small cytokines of relatively low molecular weight released from a variety of cells involved in inflammatory responses; however, the receptor on the T cells is CXCR4. The CCR5 is found on macrophages and dendritic cells.

4. **The correct answer is B.** At this stage of this patient's infection, TH cell numbers are depressed to the point that TH-dependent immune responses will be depressed. Because delayed-type hypersensitivity is a purely TH1-mediated response, it will be impaired at this stage of infection.

   Cell proliferation in response to pokeweed mitogen **(choice A)** is incorrect because pokeweed mitogen is a B-cell mitogen. These responses will remain within normal limits until the end of life in an HIV-positive patient.

   IgA production **(choice C)** is not correct because these antibodies will not begin to fall until later in the infection. IgA is a TH2-dependent antibody, but at this stage of infection, there should still be B memory cells capable of mounting this response.

   Intracellular killing of bacteria **(choice D)** is mediated by phagocytic cells. Although TH1 cytokines contribute to phagocyte activation, the intracellular killing mechanisms themselves should be unaffected in this patient.

   Rejection of allogeneic skin grafts **(choice E)** is a CD8+ cell–mediated response and should not begin to be depressed until later in the course of HIV infection. Although cytotoxic T lymphocytes are stimulated by the cytokines of TH1 cells, this response should remain relatively normal at this stage of the disease.

5. **The correct answer is D.** Most patients will begin to show immune deviation of TH responses toward the TH2 arm around 600 CD4+ cells/mm^3. As the TH2 response is augmented, IL-4 and IL-10 will be produced, which reciprocally further inhibit the TH1 response. Over time this leads to more and more profound skewing of the immune response toward a humoral response and more and more production of TH2 cytokines, such as IL-10 and IL-4.

   Interferon-γ **(choice A)** is a product of TH1 cells and stimulates the effector cells of cell-mediated immunity and reciprocally inhibits the TH2 cell. This cytokine would be decreasing in the patient at this point.

   IL-1 **(choice B)** is a product of activated macrophages. Macrophages are activated by TH1 cytokines, so interferon-γ production should begin to decrease at this time.

   IL-2 **(choice C)** is a product of many TH cells. Because it can be produced in both humoral and cell-mediated arms, its levels would probably be unaffected at this point in an infection.

   Tumor necrosis factor-β **(choice E)** is a product of TH1 cells and stimulates the effector cells of cell-mediated immunity and reciprocally inhibits the TH2 cell. This cytokine would be decreasing in the patient at this point.

Diseases Caused by Immune Responses: Hypersensitivity and Autoimmunity

13

What the USMLE Requires You to Know

- The immunologic mechanisms involved in the four types of hypersensitivity reactions
- The molecule, cell, or tissue targeted in the major autoimmune diseases

Hypersensitivity diseases are conditions in which tissue damage is caused by immune responses. They may result from uncontrolled or excessive responses against **foreign** antigens or from a **failure of self-tolerance**, in which case they are called **autoimmune diseases**. The two principal factors that determine the clinical and pathologic consequences of such conditions are the type of immune response elicited and the nature and location of the inciting antigen.

Hypersensitivity diseases are classified on the basis of the effector mechanism responsible for tissue injury, and four types are commonly recognized.

In A Nutshell

Hypersensitivity Diseases

- Excessive responses to foreign antigens
- Failure of self-tolerance (autoimmunity)

Table II-13-1. Classification of Immunologic Diseases

Type of Hypersensitivity	Immune Mechanisms	Mechanisms of Tissue Injury
Immediate (type I)	IgE	Mast cells and their mediators
Antibody-mediated (type II)	IgM and IgG Abs against cell or tissue Ags	Opsonization and phagocytosis of cells; complement- and Fc receptor–mediated recruitment and activation of neutrophils and macrophages; abnormalities in cellular functions (hormone/receptor signaling)
Immune complex-mediated (type III)	Immune complexes of circulating Ags and IgM or IgG Abs	Complement- and Fc-receptor–mediated recruitment and activation of leukocytes
T cell-mediated (type IV)	CD4+ T cells (delayed-type hypersensitivity	Macrophage activation, cytokine-mediated inflammation
	CD8+ CTLs (T-cell–mediated cytolysis)	Direct target cell killing, cytokine-mediated inflammation

In A Nutshell

In all hypersensitivity reactions:

- First exposure sensitizes.
- Subsequent exposures damage.
- Reaction is Ag-specific

In A Nutshell

Type I Hypersensitivity

- IgE-mediated
- Protective response to helminths
- Atopic/allergic individuals develop this response to inappropriate stimuli

What the hypersensitivity reactions have in common:

- The first exposure to the antigen "sensitizes" lymphocytes.
- Subsequent exposures elicit a damaging reaction.
- The response is specific to a particular antigen or a cross-reacting substance.

Type I (Immediate) Hypersensitivities

This is the only type of hypersensitivity mediated by IgE antibodies and mast cells. It is manifested within minutes of the reexposure to an antigen. The IgE response is the normal protective response against many metazoan parasites, which are too large to be phagocytized or killed by other cytopathic mechanisms. Twenty percent of all individuals in the United States, however, display this immune response against harmless environmental antigens, such as pet dander or pollen: We call these responses **atopic** or **allergic** responses.

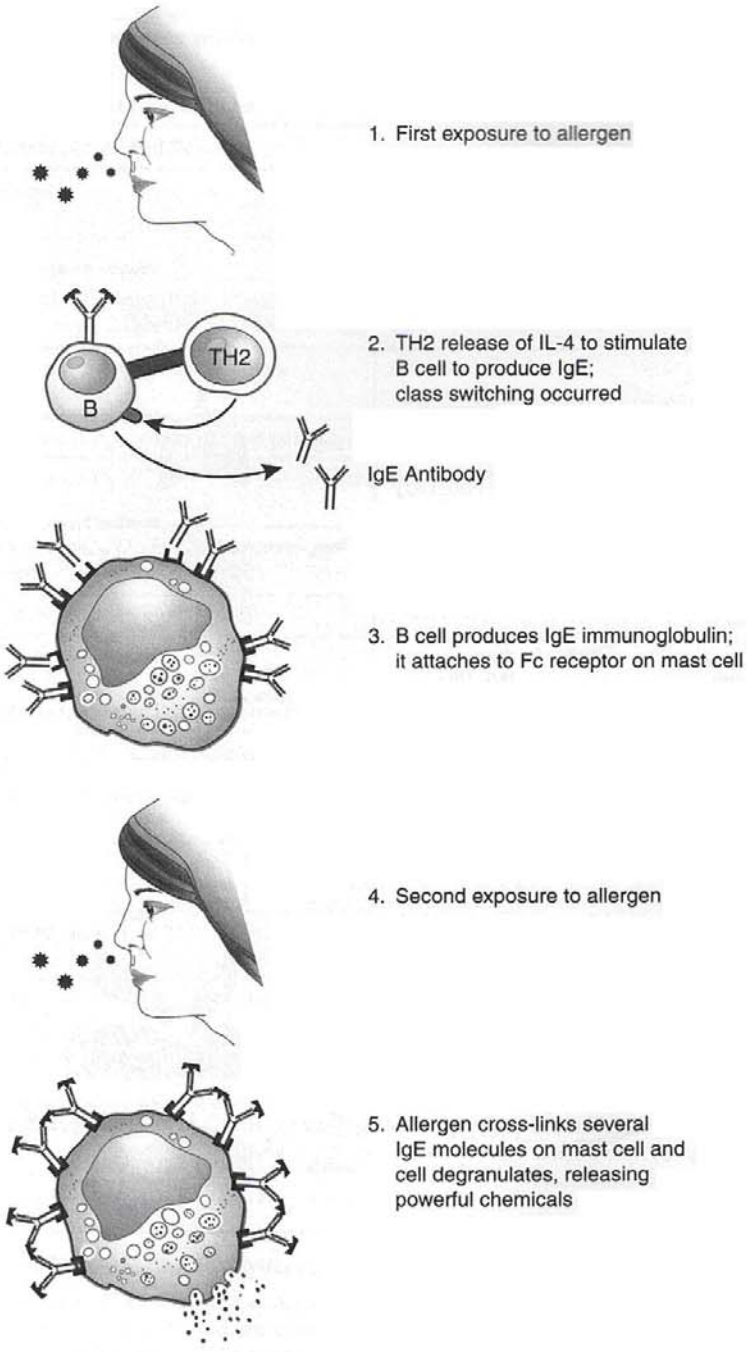

1. First exposure to allergen
TH2
B
2. TH2 release of IL-4 to stimulate B cell to produce IgE; class switching occurred
IgE Antibody
3. B cell produces IgE immunoglobulin; it attaches to Fc receptor on mast cell
4. Second exposure to allergen
5. Allergen cross-links several IgE molecules on mast cell and cell degranulates, releasing powerful chemicals

Figure II-13-1. Development of the Immediate-Hypersensitivity Reaction

In A Nutshell

Type I Effector Cells

- Mast cells
- Basophils
- Eosinophils

The effector cells of the immediate hypersensitivity reaction are mast cells, basophils, and eosinophils. The soluble substances they release into the site cause the symptoms of the reaction.

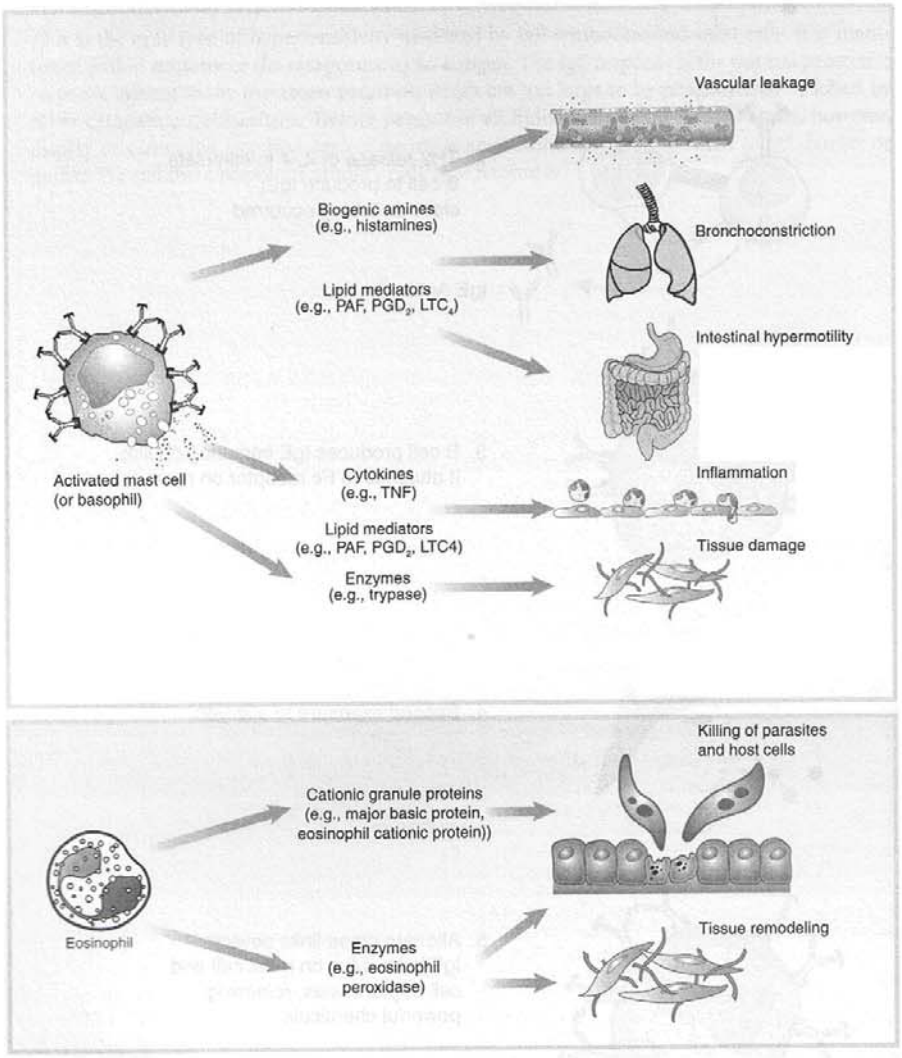


Figure II-13-2.

Two to four hours after the immediate response to release of these mediators, a **late-phase reaction** is mediated by products of the arachidonic acid cascade.

In A Nutshell

Late-phase reaction–arachidonic acid cascade

Table II-13-2. Mast Cell Mediators

Mediators Stored and Released	**Effect**
Histamine	Smooth muscle contraction; increased vascular permeability
Heparin	Anticoagulant
Eosinophil chemotactic factor A (multiple chemokines)	Chemotactic
Mediators Newly Synthesized from Arachidonic Acid	**Effect**
Prostaglandin E_2 (cyclooxygenase pathway)	Increased pain and vascular permeability
Prostaglandin D_2	Increased smooth muscle contraction and vascular permeability
Leukotrienes C_4, D_4, E_4 (lipoxygenase pathway)	Increased smooth muscle contraction and vascular permeability
Leukotriene B_4	Chemotactic for neutrophils

Table II-13-3. Allergic Diseases Due to Specific Allergens and Their Clinical Manifestations

Allergic Disease	**Allergens**	**Clinical Findings**
Allergic rhinitis (hay fever)	Trees, grasses, dust, cats, dogs, mites	Edema, irritation, mucus in nasal mucosa
Food allergies	Milk, eggs, fish, cereals, grains	Hives and gastrointestinal problems
Wheal and flare	Insect stings, in vivo skin testing for allergies	Local skin edema, reddening, vasodilation of vessels
Asthma	Inhaled materials	Bronchial and tracheal constriction, edema, mucus production, massive inflammation
Systemic anaphylaxis	Insect stings, snake venoms, drug reactions	Bronchial and tracheal constriction, complete vasodilation and death

Type II (Antibody-Mediated) Hypersensitivities

Antibodies against cell or matrix antigens cause diseases that are **specific to the tissues** where those antigens are present and are usually not systemic. In most cases, these antibodies are **autoantibodies**, but they may be produced against a foreign antigen that is cross-reactive with self components of tissues. These antibodies can cause tissue damage by three main mechanisms:

- The antibodies may opsonize cells or activate the complement system.
- The antibodies may recruit neutrophils and macrophages that cause tissue damage.
- The antibodies may bind to normal cellular receptors and interfere with their function.

In A Nutshell

Type II Hypersensitivities

- Tissue-specific autoantibodies
- Opsonize or activate complement
- Recruit inflammatory cells
- Interfere with cellular function

Table II-13-4. Type II Hypersensitivities

Disease	Target Antigen	Mechanism of Pathogenesis	Clinical Manifestations
Autoimmune hemolytic anemia	RBC membrane proteins (Rh, I Ags)	Opsonization, phagocytosis, and complement-mediated destruction of RBCs	Hemolysis, anemia
Autoimmune thrombocytopenic purpura	Platelet membrane proteins	Ab-mediated platelet destruction through opsonization and complement activation	Bleeding
Goodpasture syndrome	Noncollagenous protein in basement membranes of kidney glomeruli and lung alveoli	Complement- and Fc-receptor–mediated inflammation	Nephritis, lung hemorrhage
Acute rheumatic fever	Streptococcal cell-wall Ag; Ab cross-reacts with myocardial Ag	Inflammation, macrophage activation	Myocarditis, arthritis
Myasthenia gravis	Acetylcholine receptor	Ab inhibits acetylcholine binding, downmodulates receptors	Muscle weakness, paralysis
Graves disease	TSH receptor	Ab-mediated stimulation of TSH receptors	Hyperthyroidism followed by hypothyroidism
Type II (non-insulin–dependent) diabetes	Insulin receptor	Ab inhibits binding of insulin	Hyperglycemia, ketoacidosis
Pernicious anemia	Intrinsic factor of gastric parietal cells	Neutralization of intrinsic factor, decreased absorption of vitamin B_{12}	Abnormal erythropoiesis, anemia

In some types of type II hypersensitivities, complement is activated and/or ADCC is active (e.g., hemolytic disease of the newborn). In other types of type II hypersensitivities, cell function is altered in the absence of complement activation and ADCC (e.g., myasthenia gravis and Graves disease).

An important example of type II hypersensitivity is hemolytic disease of the newborn (HDNB), also known as erythroblastosis fetalis. In the fetus, this disease is due to transport of IgG specific for one of the Rhesus (Rh) protein antigens (RhD) across the placenta. About 85% of people are Rh+. If a pregnant woman is Rh– and the father is Rh+, there is a chance that the fetus will also be Rh+. This situation will pose no problem in the first pregnancy, as the mother's immune system will not usually encounter fetal blood cell antigens until placental separation at

the time of birth. At that time, however, Rh+ fetal red blood cells will enter the maternal circulation and stimulate a T-dependent immune response, eventually resulting in the generation of memory B cells capable of producing IgG antibody against RhD. In a subsequent pregnancy with another Rh+ fetus, this maternal IgG can be transported across the placenta, react with fetal Rh+ red cells, and activate complement, producing hemolytic disease. Hemolytic disease of the newborn can be prevented by treating the Rh– mother with RhoGAM™, a preparation of human anti-RhD IgD antibody, at 28 weeks of gestation and again with a human anti-RhD IgG antibody within 72 hours after birth. This antibody effectively eliminates the fetal Rh+ cells before they can generate RhD-specific memory B cells in the mother. Anti-RhD antibody should be given to any Rh– individual following any termination of pregnancy.

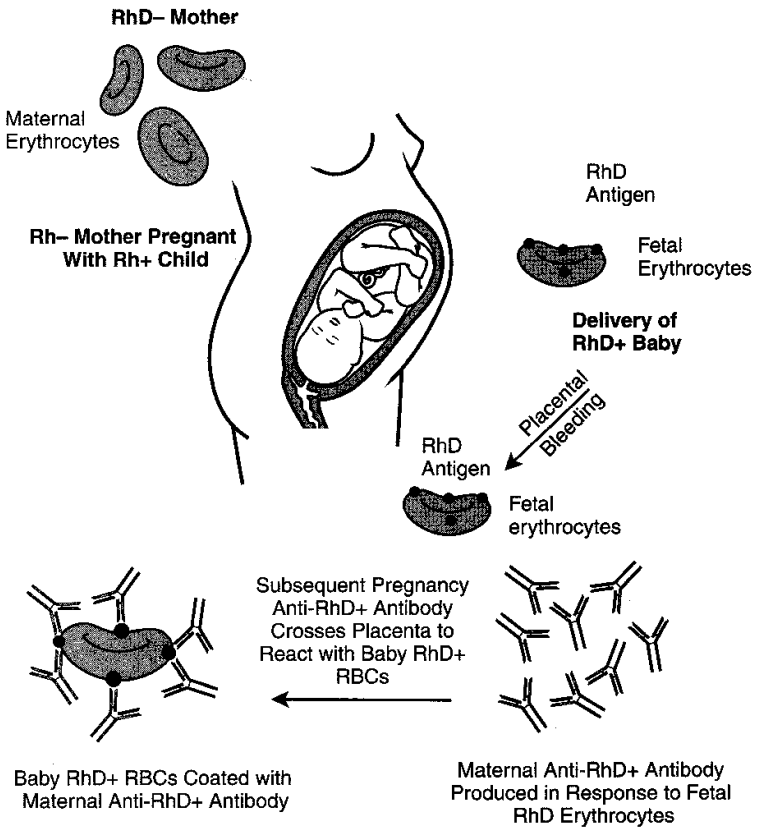

RhD– Mother
Maternal Erythrocytes
Rh– Mother Pregnant With Rh+ Child
RhD Antigen
Fetal Erythrocytes
Delivery of RhD+ Baby
Placental Bleeding
RhD Antigen
Fetal erythrocytes
Subsequent Pregnancy Anti-RhD+ Antibody Crosses Placenta to React with Baby RhD+ RBCs
Baby RhD+ RBCs Coated with Maternal Anti-RhD+ Antibody
Maternal Anti-RhD+ Antibody Produced in Response to Fetal RhD Erythrocytes

Figure II-13-3. Hemolytic Disease of the Newborn

In A Nutshell

Hemolytic Disease of the Newborn (HDNB)

- Erythroblastosis fetalis
- Anti-RhD+ IgG
- Formed in Rh– mother carrying Rh+ child
- First pregnancy sensitizes
- Ab crosses placenta and injures subsequent fetuses
- Prevent with RhoGAM™

In A Nutshell

Graves Disease

- Anti-TSH receptor antibodies
- Hyperthyroidism early
- Hypothyroidism late

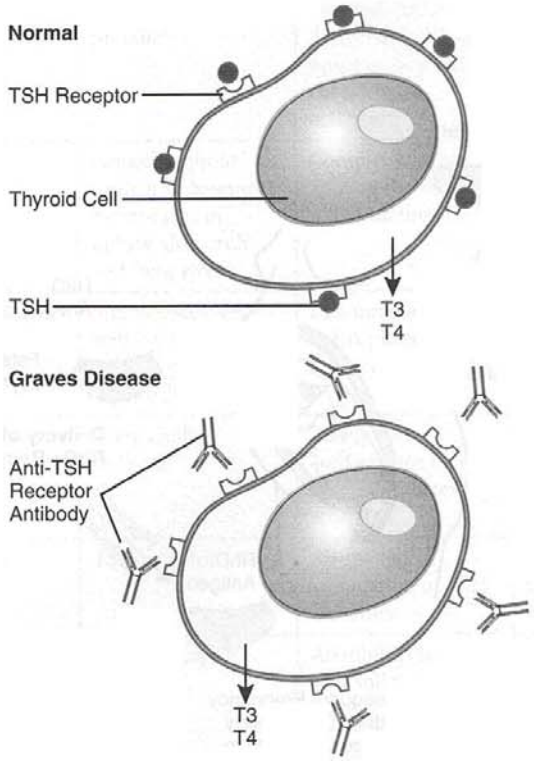


Figure II-13-4. Antibodies to the TSH Receptor in Hyperthyroidism

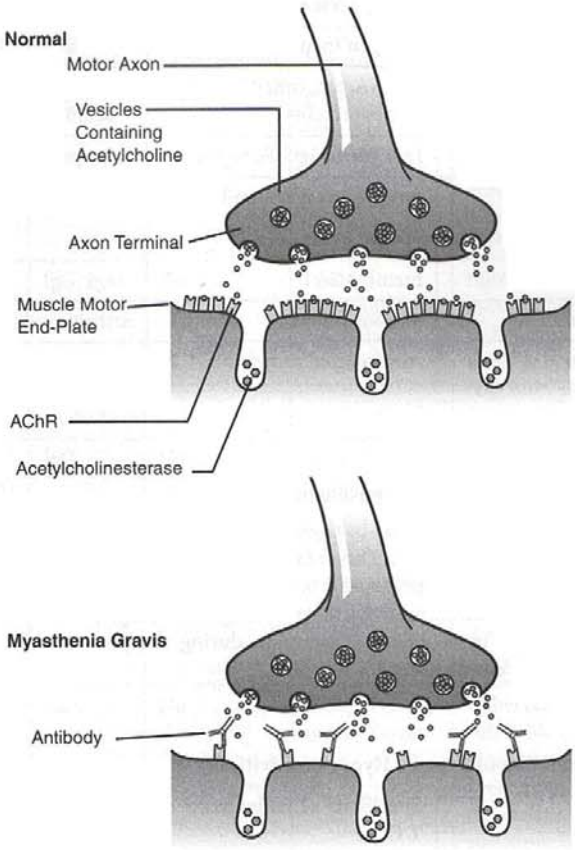


Figure II-13-5. Antibodies Against Acetylcholine Receptors in Myasthenia Gravis

Type III (Immune Complex) Hypersensitivities

The immune complexes that cause disease may either involve self or foreign antigens bound to antibodies. These immune complexes are filtered out of the circulation in the small vasculature, so their sites of ultimate damage do not reflect their sites of origin. These diseases tend to be **systemic**, with little tissue or organ specificity.

In A Nutshell

Type III Hypersensitivities

- Systemic damage
- Immune complexes activate complement
- Self or foreign Ags

Table II-13-5. Examples of Type III Hypersensitivities

Disease	Antigen Involved	Clinical Manifestations
Systemic lupus erythematosus	dsDNA, Sm, other nucleoproteins	Nephritis, arthritis, vasculitis, rash
Rheumatoid arthritis	IgM versus IgG Fc region	Joint pain, erosions
Poststreptococcal glomerulonephritis	Streptococcal cell wall Ags (may be "planted" in glomerular basement membrane)	Nephritis
Serum sickness	Various proteins	Arthritis, vasculitis, nephritis
Arthus reaction	Any injected protein	Local pain and edema

In A Nutshell

Type IV Hypersensitivities

- Delayed-type (48 to 72 hours)
- CD4+ TH cells mediate
- Activate macrophages
- Cause inflammation
- Common in chronic intracellular infections

Type IV (T-Cell–Mediated) Hypersensitivities

T lymphocytes may cause tissue injury by triggering delayed-type hypersensitivity (DTH) reactions or by directly killing target cells. These reactions are elicited by CD4+ TH1 cells and CD8+ cells, which secrete cytokines that activate macrophages (IFN-γ) and induce inflammation (TNF). These T cells may be autoreactive or specific against foreign protein antigens bound to tissues. T-cell–mediated tissue injury is common during the protective immune response against persistent intracellular microbes.

Table II-13-6. Examples of Type IV Hypersensitivities

Disease	Specificity of Pathogenic T Cells	Clinical Manifestations
Insulin-dependent diabetes mellitus (type I)*	Islet-cell antigens, insulin, glutamic acid decarboxylase, others	Polydipsia, polyuria, polyphagia, ketoacidosis
Multiple sclerosis	Myelin basic protein, proteolipid protein	Progressive demyelination, blurred vision, paralysis
Contact dermatitis	Nickel, poison ivy/oak catechols, hapten/carrier	Vesicular skin lesions, pruritus, rash
Peripheral neuritis	P2 protein of peripheral nerve myelin	Ascending paralysis
Guillain-Barré syndrome*	Peripheral nerve myelin or gangliosides	Ascending paralysis, peripheral nerve demyelination
Hashimoto's thyroiditis*	Unknown Ag in thyroid	Hypothyroidism

*Diseases classified at type IV pathologies in which autoantibodies are present and used as clinical markers

Table II-13-7. Hypersensitivity Summary

Type	Antibody	Complement	Effector Cells	Examples*
I (Immediate)	IgE	No	Basophil, mast cell	Hay fever, atopic dermatitis, **insect venom sensitivity**, anaphylaxis to drugs, some food allergies, allergies to animals and animal products, **asthma**
II (Cytotoxic)	IgG, IgM	Yes	PMN, macrophages, NK cells	Autoimmune or drug-induced hemolytic anemia, transfusion reactions, **HDNB**, hyperacute graft rejection, Goodpasture disease, **rheumatic fever**
II (Noncytotoxic)	IgG	No	None	**Myasthenia gravis**, **Graves disease**, type 2 diabetes mellitus
III (Immune complex)	IgG, IgM	Yes	PMN, macrophages	**SLE**, **RA**, polyarteritis nodosa, poststreptococcal glomerulonephritis, Arthus reaction, serum sickness
IV (Delayed, DTH)	None	No	CTL, TH1, macrophages	**Tuberculin test**, tuberculosis, leprosy, Hashimoto thyroiditis, poison ivy (**contact dermatitis**), acute graft rejection, **GVHD**, **IDDM**

*Most high-yield diseases are bolded.

Abbreviations: HDNB, hemolytic disease of the newborn; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis; GVHD, graft-versus-host disease; IDDM, insulin-dependent diabetes mellitus

The Pathogenesis of Autoimmunity

The key factor in the development of autoimmunity is the recognition of self-antigens by autoreactive lymphocytes, which then become activated, proliferate, and differentiate to produce effector cells and cytokines that cause tissue injury. Autoimmunity must initially result from a failure of mechanisms of **self-tolerance,** as cells are "educated" in the bone marrow and thymus. Genetic factors contribute to this susceptibility, as well as environmental and hormonal triggers. Infections and tissue injury may alter the way that self-antigens are presented to lymphocytes and serve as an inciting factor in the development of disease. Because autoimmune reactions against one self-antigen may injure other tissues, exposing other potential self-antigens for recognition, autoimmune diseases tend to be chronic and progressive.

Among the strongest genetic associations with the development of autoimmune disease are the **class II MHC genes.**

In A Nutshell

Autoimmunity

- Failure of self-tolerance
- Genetics (class II MHC)
- Environment (infections)
- Hormones

Table II-13-8. Examples of HLA-Linked Immunologic Diseases

Disease	HLA Allele
Rheumatoid arthritis	DR4
Insulin-dependent diabetes mellitus	DR3/DR4
Multiple sclerosis	DR2
Systemic lupus erythematosus	DR2/DR3
Ankylosing spondylitis	B27
Celiac disease	DQ2 or DQ8

In A Nutshell

Infections can trigger autoimmunity through:

- Activating bystanders
- Molecular mimicry
- Inflammatory damage

Infections may trigger autoimmune responses by:

- **Bystander activation**—immune responses may recruit leukocytes and increase expression of costimulators, which activate T lymphocytes that are not specific for the infectious pathogen.
- **Molecular mimicry**—antigens of a microbe cross-react with or mimic self antigens.
- **Inflammation** and associated damage may expose self antigens that are normally concealed from immune cells.

Strategies for the Therapy of Hypersensitivity and Autoimmune Diseases

The mainstay of therapy for hypersensitivity diseases is directed at modification of T-cell function:

- Inhibit proliferation (cyclosporine)
- Inhibit function (corticosteroids)
- Kill T cells (cyclophosphamide)
- Antagonists to proinflammatory cytokines or costimulatory molecules (monoclonals or binding proteins)

In A Nutshell

Therapies for immune diseases:

- Inhibit proliferation
- Inhibit function
- Kill T cells
- Antagonize damaging products

These drugs are directed toward reducing tissue injury due to the effector phases of these responses. Other therapeutic regimens being used to inhibit pathologic responses include:

- Injection of small doses of allergens to reduce specific IgE production over time. The mechanism through which this procedure works is not clear. One theory is that increasing small doses of antigen leach preformed IgE out of the tissues. Another is that continuous small-dose exposures may stimulate TH cell populations that do not favor further IgE production.
- Plasmapheresis may be used to decrease circulating levels of antibody in diseases caused by antibodies or immune complexes.
- High doses of intravenous IgG may bind to Fc receptors and inhibit antibody synthesis.
- Attempts to induce tolerance by high-dose administration of antigens or altered forms of self antigens (myelin basic protein).

In A Nutshell

Immunologic Therapies

- Desensitization
- Plasmapheresis
- Intravenous IgG
- Tolerance induction

Chapter Summary

- There are four types of hypersensitivities: immediate, antibody-mediated (cytotoxic, blocking, enhancing), immune complex–mediated and T cell–mediated.
- Hypersensitivity reactions require initial sensitization, and subsequent exposures to the same or cross-reactive antigens cause the damage.
- Type I hypersensitivities (immediate) involve IgE antibodies and mast cells, show symptoms in minutes, and are mounted against harmless environmental antigens in atopic or allergic individuals.
- Initial tissue damage in immediate hypersensitivities is due to release of mast cell mediators, and late-phase reactions involve products of the arachidonic acid cascade.
- Examples of type I hypersensitivities include hay fever, asthma, food allergies, and systemic anaphylaxis.
- Type II (antibody-mediated) hypersensitivities are tissue specific and involve autoantibodies that opsonize or activate complement. Some noncytotoxic forms (myasthenia gravis, Graves disease, type II diabetes) cause interference with cellular function.
- Examples of cytotoxic type II hypersensitivities include autoimmune hemolytic anemia, hemolytic disease of the newborn, autoimmune thrombocytopenic purpura, Goodpasture syndrome, rheumatic fever, and pernicious anemia.
- Type III (immune complex) hypersensitivities cause systemic damage by activating complement wherever immune complexes of antibodies against self or foreign antigens are filtered from the circulation.
- Examples of type III hypersensitivities include systemic lupus erythematosus, rheumatoid arthritis, poststreptococcal glomerulonephritis, serum sickness, and the Arthus reaction.
- Type IV hypersensitivities are delayed-type (manifesting symptoms in 48 to 72 hours after reexposure); are caused by TH1 cells, CD8+ cells, and macrophages; and are common results of infection with persistent intracellular microbes.
- Examples of type IV hypersensitivities include insulin-dependent diabetes mellitus, multiple sclerosis, contact dermatitis, Guillain-Barré syndrome, and Hashimoto thyroiditis.
- Autoimmune diseases may associate with specific class II MHC haplotypes, environmental factors, or hormonal factors.
- Therapies for immune diseases involve inhibition of proliferation or function of T cells, killing of T cells, or antagonism of the damaging products of T cells.

Review Questions

1. A 43-year-old woman is seen by her physician with complaints of painful, swollen joints. On examination, her hands appear to be disfigured at the joints with apparent subcutaneous nodule formation. Her left knee is grossly enlarged, and 100 mL of fluid is withdrawn from the joint capsule. An examination of this fluid should reveal which of the following?

   (A) Activated T lymphocytes
   (B) Antibodies against type IV collagen
   (C) Antibodies against double-stranded DNA
   (D) Antibodies against microsomal antigens
   (E) IgM antibodies reactive with the Fc region of IgG

2. A 36-year-old farmer has been exposed to poison ivy on several different occasions, and he usually gets very severe skin lesions. A pharmaceutical company is developing cytokines by recombinant DNA technology and formulating them in a fashion that they are readily absorbed through the skin. Which of the following cytokines administered topically could inhibit the severity of this reaction?

   (A) γ-Interferon
   (B) IL-2
   (C) IL-3
   (D) IL-8
   (E) IL-10

3. In the 1960s, it was quickly ascertained that Peace Corps workers sent to schistosome-endemic areas were exposed to massive initial doses of cercariae before any protective immunity existed. In these individuals, IgG antibodies developed in response to the developing worms, and when the adults began their prodigious release of eggs into the circulation, the patients suffered acute and potentially life-threatening symptoms of fever, edema, arthralgia, and rash. Which of the following is another condition that arises by a similar immunologic mechanism?

   (A) Arthus reaction
   (B) Atopic allergy
   (C) Goodpasture syndrome
   (D) Tuberculin reaction
   (E) Transfusion reaction

4. A young, newly married woman goes to her physician with concerns about a "hereditary problem" described to her by her mother that was associated with her own birth in 1968. Her family was poor, and her mother received no medical prenatal care before she was born "blue" and covered with "splotches" and "bruises." Although an earlier sibling had been born apparently normal, the patient required multiple transfusions before her condition stabilized, and two further pregnancies of her mother ended in stillbirths. The patient is concerned about the potential for development of similar problems in her own pregnancies. Blood tests ordered by the physician confirm his suspicions. How should he advise this patient?

   (A) Her husband should be tested for Rh incompatibility
   (B) She is RhD– and should be treated postpartum with RhoGAM™
   (C) She is RhD–; there is no risk to a fetus
   (D) She is RhD+ and should be treated postpartum with RhoGAM™
   (E) She is RhD+; there is no risk to a fetus

5. In native Egyptian populations, children are exposed to the cercariae of the fluke *Schistosoma mansoni* in early childhood when they wade in irrigation ditches throughout the Nile Delta. On first exposure, the cercariae penetrate the skin and become schistosomula, which enter the circulation and eventually mature in the mesenteric veins. On subsequent exposures, schistosomula are frequently killed within minutes by an immune response in the skin manifested by intense itching, stinging, and urticaria. What is this protective immune response a manifestation of?

   (A) Arthus reaction
   (B) Contact dermatitis
   (C) Passive cutaneous anaphylaxis
   (D) Serum sickness
   (E) Type I hypersensitivity

Answers and Explanations

1. **The correct answer is E.** This woman has the signs of rheumatoid arthritis, which is an autoimmune disease characterized by the development of IgM antibodies against one's own IgG (rheumatoid factor). These antibodies complex with their antigen (IgG) and precipitate out of the blood in the joints and vasculature, where they cause complement activation. Complement lysis is responsible for the damage to the tissues. This is an example of an immune complex–mediated (type III) hypersensitivity reaction.

   Activated T lymphocytes (**choice A**) would be found in the circulation in some cases of infection, but are not a major feature of the type III hypersensitivities. In multiple sclerosis, which is a T-cell–mediated autoimmune disease, activated T lymphocytes sensitized to myelin can be found in the cerebrospinal fluid.

   Antibodies against type IV collagen (**choice B**) would be found in Goodpasture disease. This is an example of a type II (cytotoxic antibody) hypersensitivity. In this disease, linear deposition of IgG and complement occurs in the alveolar and glomerular basement membranes and causes destruction of the underlying tissues.

   Antibodies against double-stranded DNA (**choice C**) occur in the plasma of patients with systemic lupus erythematosus. This is a type III hypersensitivity disease, like rheumatoid arthritis, so the underlying principle of pathogenesis is similar, but patients with lupus have a characteristic facial rash, and symptoms would have their onset between 20 and 40 years of age. Although SLE can be associated with arthritis, it is not generally disfiguring as described here.

   Antibodies against microsomal antigens (**choice D**) and thyroglobulin would be found in Hashimoto thyroiditis. In addition, T cells sensitized to thyroid antigens are found, so this disease is generally categorized as a type IV (delayed-type) hypersensitivity response. The age group and sex of the patient are correct for this diagnosis, but the symptoms would include hyperthyroidism, not joint inflammation.

2. **The correct answer is E.** IL-10 is produced by TH2 cells and inhibits TH1 cells. Because the response to poison ivy is a delayed-type hypersensitivity response and therefore is mediated by TH1 cells and macrophages, inhibiting their activity would minimize the severity of the reaction.

   γ-Interferon (**choice A**) is a product of TH1 cells, CTLs, and NK cells. It inhibits the proliferation of TH2 cells and therefore would skew the immune response toward a more potent cell-mediated arm. This is not a cytokine that would help this patient: It would make his condition worse.

   IL-2 (**choice B**) is a product of TH cells that induces the proliferation and enhances the activity of antigen-primed TH cells and CTLs. It would tend to increase the symptoms of this patient, not ameliorate them.

   IL-3 (**choice C**) is a product of TH cells and NK cells. It acts on hematopoietic cells to encourage myeloid cell development. It would neither hinder nor help this man's condition.

   IL-8 (**choice D**) is a product of macrophages and endothelial cells and acts on neutrophils to attract them to areas of inflammation. It would neither hinder nor help resolve the symptoms of a delayed-type hypersensitivity response.

3. **The correct answer is A.** The condition described here is an immune complex–mediated pathology. When large amounts of antigen are added into a situation where there is preexistence of a large amount of antibody, the precipitation of those complexes in the small vasculature causes a type III hypersensitivity response. The only syndrome on this list that also has a type III etiology is the Arthus reaction.

Atopic allergy (**choice B**) is a type I hypersensitivity, mediated by IgE antibodies and mast cells. Although many parasitic diseases elicit IgE and ADCC by eosinophils, the question stem clearly stipulates the presence of IgG antibodies, so a type I hypersensitivity reaction is ruled out.

Goodpasture syndrome (**choice C**) is an example of a type II, cytotoxic antibody hypersensitivity response. In these cases, antibody binds to cells or tissues of the body and elicits complement activation in those locations. The result is a tissue- or organ-specific pathology, and not a systemic problem as described here.

The tuberculin reaction (**choice D**) is a type IV hypersensitivity response. Delayed-type hypersensitivities are mediated by TH1 cells and have no contribution whatsoever from antibodies.

The transfusion reaction (**choice E**) is an example of a type II, cytotoxic antibody hypersensitivity response. In these cases, antibody binds to cells or tissues of the body and elicits complement activation in those locations. The result is a tissue- or organ-specific pathology, and not a systemic problem as described here.

4. **The correct answer is E.** From the description in the vignette, this patient was born with hemolytic disease of the newborn, or erythroblastosis fetalis. This occurs when an Rh– woman becomes pregnant with an Rh+ fetus. During a first pregnancy (the patient's older sibling), no problems are encountered, but the mother is sensitized against the RhD antigens. In all subsequent pregnancies, anti-Rh IgG antibodies from the mother can cross the placenta and cause hemolysis of the child's RBCs. This means that the patient is RhD+. It is irrelevant what the Rh type of any fetus she would carry is: She will make no immunologic response.

   That her husband should be tested for Rh incompatibility (**choice A**) is not correct because the patient is Rh+. She will not recognize any Rh product as foreign.

   That she is RhD– and should be treated postpartum with RhoGAM™ (**choice B**) is not true because she is RhD+. Women who are RhD– should be treated with RhoGAM (IgD or IgG anti-Rh antibodies) during and following the termination of any pregnancy.

   That she is RhD–; there is no risk to a fetus (**choice C**) is not true because she is RhD+. Women who are RhD– should be treated with RhoGAM (IgD or IgG anti-Rh antibodies) during and following the termination of any pregnancy.

   That she is RhD+ and should be treated postpartum with RhoGAM (**choice D**) is not true because there is no need to treat RhD+ women in any way following termination of pregnancies.

5. **The correct answer is E.** The description in the vignette is a type I hypersensitivity reaction—the only type of hypersensitivity that can be manifested in minutes. These are IgE-mediated responses and are an important protective response against helminth parasites that migrate through the tissues.

   The Arthus reaction (**choice A**) is an example of a type III (immune complex–mediated) pathology. These hypersensitivities develop when antigen is added to pre-existing antibody and immune complexes are filtered out of the circulation in the small vasculature. Complement is activated in these locations, and the underlying tissue is damaged. In this vignette, the killing is occurring in the skin and is associated within minutes with stinging, itching, and urticaria.

   Contact dermatitis (**choice B**) is an example of a type IV (delayed-type) hypersensitivity response. After the sensitizing exposure, symptoms of this type of hypersensitivity will occur in 48 to 72 hours (not minutes, as described here).

   Passive cutaneous anaphylaxis (**choice C**) is an example of a type I hypersensitivity reaction, but it is used to diagnose these conditions using passive transfer of serum. It is not a mechanism of protection, but a diagnostic technique.

Serum sickness (**choice D**) is an example of a type III (immune complex-mediated) pathology. These hypersensitivities develop when antigen is added to pre-existing antibody and immune complexes are filtered out of the circulation in the small vasculature. Complement is activated in these locations, and the underlying tissue is damaged. In this vignette, the killing is occurring in the skin and is associated within minutes with stinging, itching, and urticaria.

Transplantation Immunology

14

What the USMLE Requires You to Know

- The different types of tissue transplantation performed in medicine
- The mechanisms and timing of graft rejection phenomena
- The pathogenesis of graft-versus-host disease
- The techniques for tissue compatibility testing
- The therapeutic strategies to inhibit graft rejection

Transplantation is the process of taking cells, tissues, or organs (a **graft**) from one individual (the **donor**) and implanting them into another individual or another site in the same individual (the **host** or **recipient**). **Transfusion** is a special case of transplantation and the most frequently practiced today, in which circulating blood cells or plasma are infused from one individual into another. As we have seen in previous chapters, the immune system is elaborately evolved to recognize minor differences in self antigens that reflect the invasion of harmful microbes or pathologic processes, such as cancer. Unfortunately, it is this same powerful mechanism of self-protection that thwarts tissue transplantation because tissues derived from other individuals are recognized as **"altered-self"** by the educated cells of the host's immune system.

In A Nutshell

Transplantation

- Tissues taken from donor given to host
- Transfusion most common

Several different types of grafts are used in medicine:

- Autologous grafts (or **autografts**) are those where tissue is moved from one location to another in the same individual (skin grafting in burns or coronary artery replacement with saphenous veins).
- **Syngeneic** grafts are those transplanted between genetically identical individuals (monozygotic twins).
- **Allogeneic** grafts are those transplanted between genetically different members of the same species (kidney transplant).
- **Xenogeneic** grafts are those transplanted between members of different species (baboon heart into human child).

In A Nutshell

Types of Grafts

- Autografts
- Syngeneic grafts
- Allogeneic grafts
- Xenogeneic grafts

Note

MHC alleles are expressed codominantly.

The recognition of transplanted cells as self or foreign is determined by the extremely polymorphic genes of the major histocompatibility complex, which are expressed in a **codominant** fashion. This means that each individual inherits a complete set or **haplotype** from each parent and virtually assures that two genetically unrelated individuals will have distinctive differences in the antigens expressed on their cells. The net result is that all grafts except autografts are ultimately identified as foreign invading proteins and destroyed by the process of **graft rejection.** Even syngeneic grafts between identical twins can express recognizable antigenic differences due to somatic mutations that occur during the development of the individual. For this reason, all grafts except autografts must be followed by some degree of lifelong immunosuppression of the host to attempt to avoid rejection reactions.

In A Nutshell

Graft Rejection Effectors

- CTLs
- Macrophages
- Ab-mediated

The time sequence of allograft rejection differs depending on the tissue involved but always displays specificity and memory. As the graft becomes vascularized, CD4+ and CD8+ cells that migrate into the graft from the host become sensitized and proliferate in response to both major and minor histocompatibility differences. In the **effector phase** of the rejection, TH cytokines play a critical role in stimulating macrophage, cytotoxic T cell, and even antibody-mediated killing. Interferons and TNF-α and -β all increase the expression of class I MHC molecules in the graft, and IFN-γ increases the expression of class II MHC as well, increasing the susceptibility of cells in the graft to MHC-restricted killing.

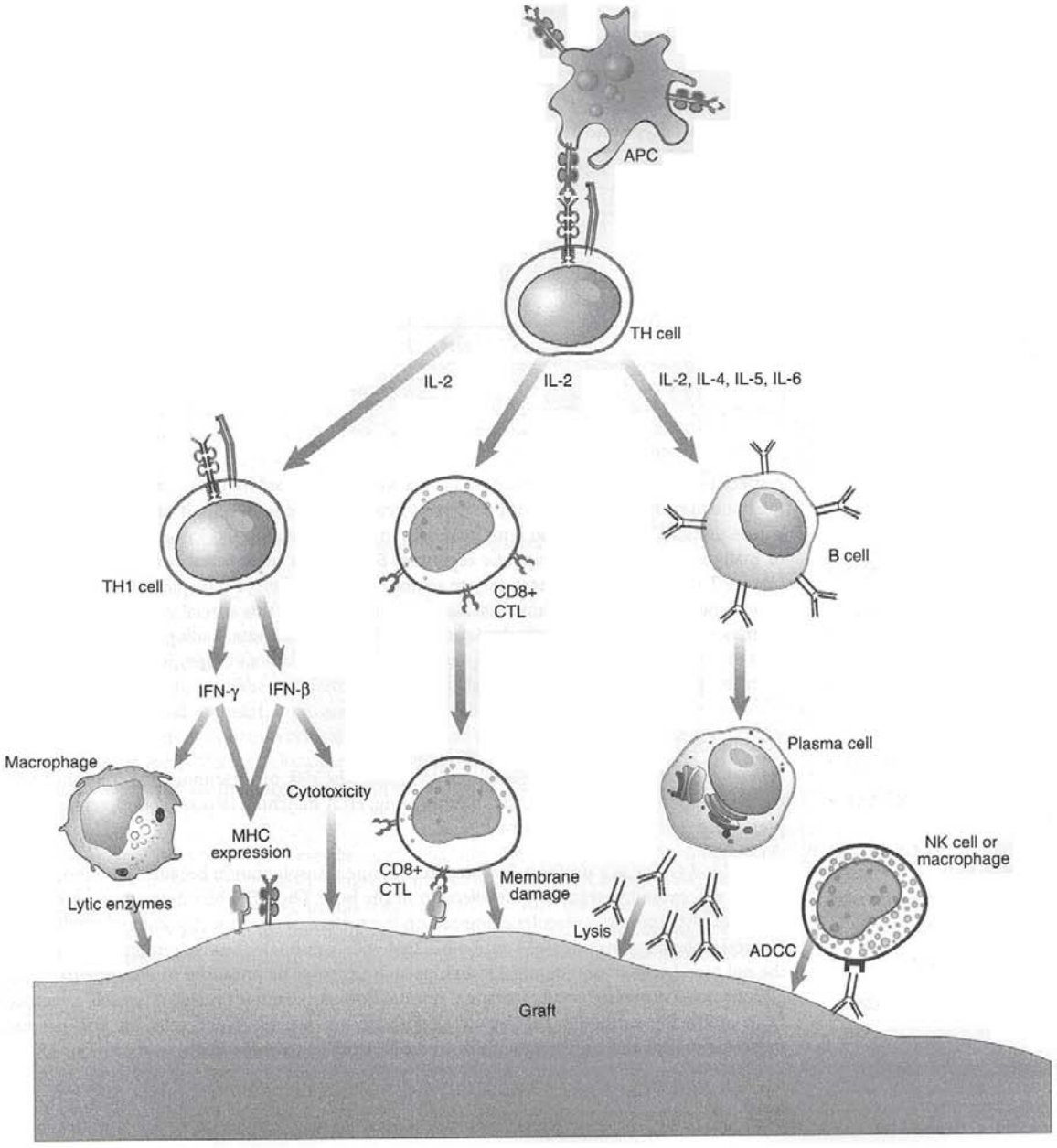


Figure II-14-1. Mechanism of Graft Rejection

In A Nutshell

Graft Rejection

- Hyperacute
- Accelerated
- Acute
- Chronic

Four different classes of allograft rejection phenomena are classified according to their time of activation and the type of effector mechanism that predominates.

Table II-14-1. Type and Tempo of Rejection Reactions

Type of Rejection	Time Taken	Cause
Hyperacute	Minutes to hours	Preformed antidonor antibodies and complement
Accelerated	Days	Reactivation of sensitized T cells
Acute	Days to weeks	Primary activation of T cells
Chronic	Months to years	Causes are unclear: antibodies, immune complexes, slow cellular reaction, recurrence of disease

In A Nutshell

Graft-Versus-Host Disease

- Bone marrow transplant
- Grafted T cells attack host
- Rash, jaundice, diarrhea, gastrointestinal hemorrhage

A special case of tissue transplantation occurs when the grafted tissue is bone marrow. Because the bone marrow is the source of pluripotent hematopoietic stem cells, it can be used to reconstitute myeloid, erythroid, and lymphoid cells in a recipient who has lost these cells as a result of malignancy or chemotherapeutic regimens. Because the bone marrow is a source of some mature T lymphocytes, it is necessary to remove these cells before transplantation to avoid the appearance of **graft-versus-host disease** in the recipient. In this special case of rejection, any mature T cells remaining in the bone marrow inoculum can attack allogeneic MHC-bearing cells of the recipient and cause widespread epithelial cell death accompanied by rash, jaundice, diarrhea, and gastrointestinal hemorrhage.

Testing for Tissue Compatibility

Several clinical laboratory tests are used to reduce the risk of immunologic rejection during transplantation. These include ABO blood typing, HLA matching (tissue typing), screening for preformed antibodies, and crossmatching.

In A Nutshell

Tissue Compatibility Testing

- ABO blood typing
- Mixed lymphocyte reaction (class II)
- Microcytotoxicity test (class I)

ABO blood typing is a uniform first step in all tissue transplantation because ABO incompatibilities will cause hyperacute graft rejection in the host. The ABO blood group antigens are a group of glycoprotein molecules expressed on the surface of erythrocytes and endothelial cells. Natural isohemagglutinins (IgM antibodies that will agglutinate the glycoprotein molecules on the red blood cells of nonidentical individuals) are apparently produced in response to similar glycoproteins expressed on the intestinal normal flora. A person is protected (by self-tolerance) from producing antibodies that would agglutinate his own red blood cells, but will produce those agglutinins that will react with the red cells from other individuals.

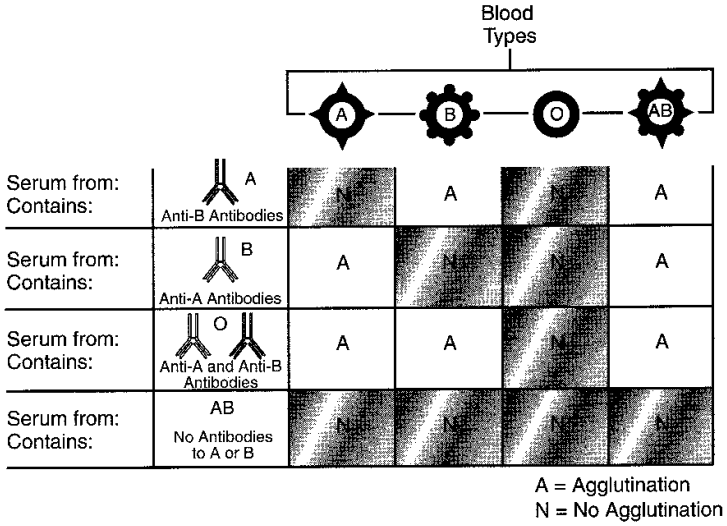


Figure II-14-2. Agglutination Test for Blood Typing

In cases where a living donor may be used for tissue transplantation (kidney and bone-marrow transplants), **tissue typing** to match the HLA antigens of recipient and donor is the next step. In general, the larger the number of matched MHC alleles between donor and recipient, the better the graft survival. In heart, lung, and liver transplantation, this step is often not considered because recipients are often in critical condition, and these organs cannot be stored indefinitely while tests are performed. Routine HLA typing focuses only on **HLA-A, HLA-B, and HLA-DR** because these are the only loci that appear to predict the likelihood of rejection of the transplant.

Two different laboratory techniques may be used to identify the histocompatibility antigens of donor and recipient. To identify class I antigens, a microcytotoxicity test using antisera against specific class I antigens is performed. In this test, lymphocytes from the donor or recipient are mixed with different antisera. If the antibodies recognize their specific epitope on the cells, they will be bound there, and addition of complement will result in cell lysis. The lysis of cells is monitored by adding a dye that will penetrate cells whose membranes have become leaky from the actions of complement.

In A Nutshell

Routine HLA Typing

- HLA-A
- HLA-B
- HLA-DR

In A Nutshell

Class I compatibility testing–microcytotoxicity test

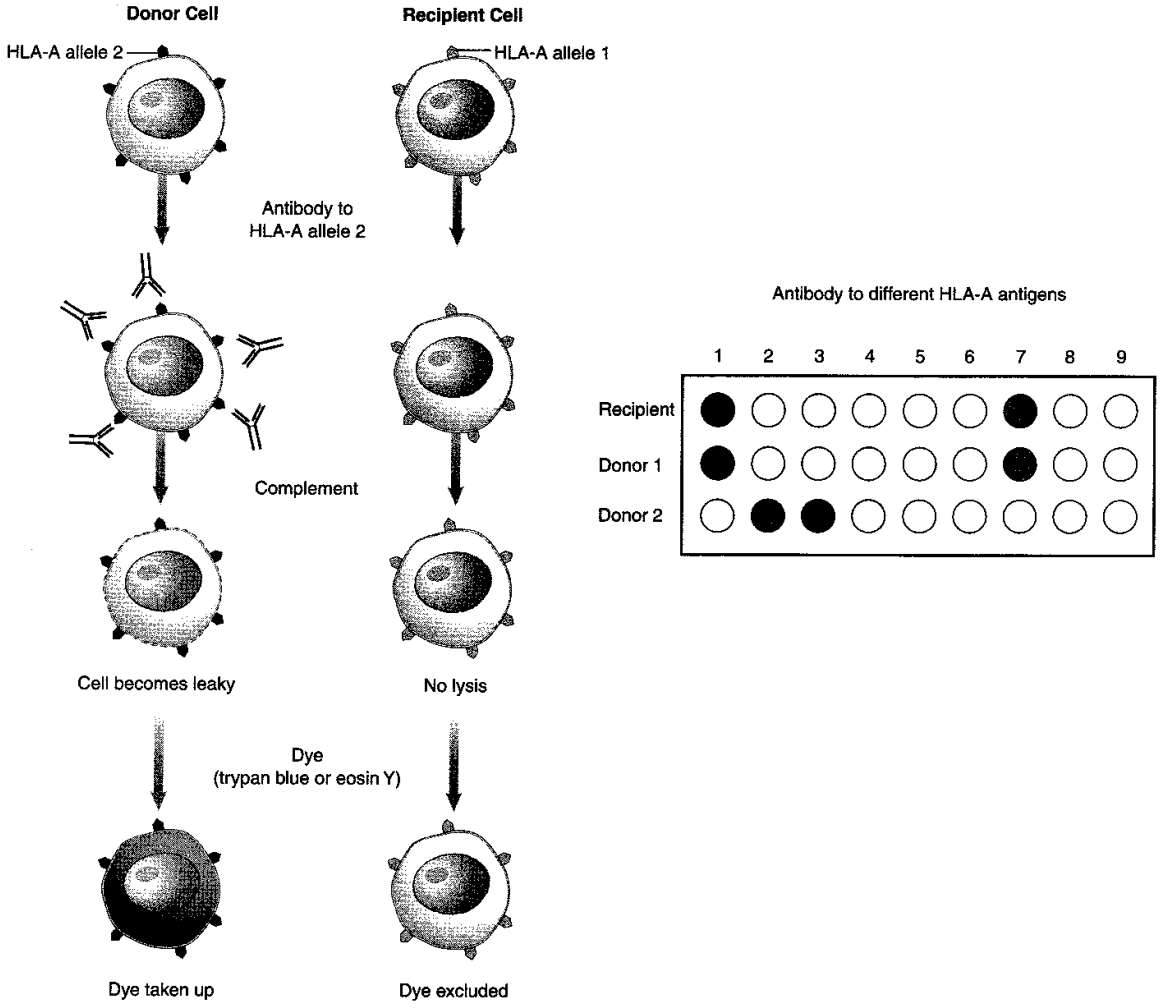


Figure II-14-3. Microcytotoxicity Assay

In A Nutshell

Class II compatibility testing–mixed lymphocyte reaction

To identify **class II antigens**, a microcytotoxicity test (*see* Figure 14-3) or the **mixed lymphocyte reaction** (MLR) may be performed. In the MLR, lymphocytes from one individual being tested are irradiated so that they cannot proliferate, but will act as **stimulator cells** for the presentation of MHC antigens. The other individual's cells are added to the culture, and uptake of tritiated thymidine is used as an indicator of cell proliferation. If the MHC class II antigens are different, proliferation will occur. If they are the same, no proliferation will occur.

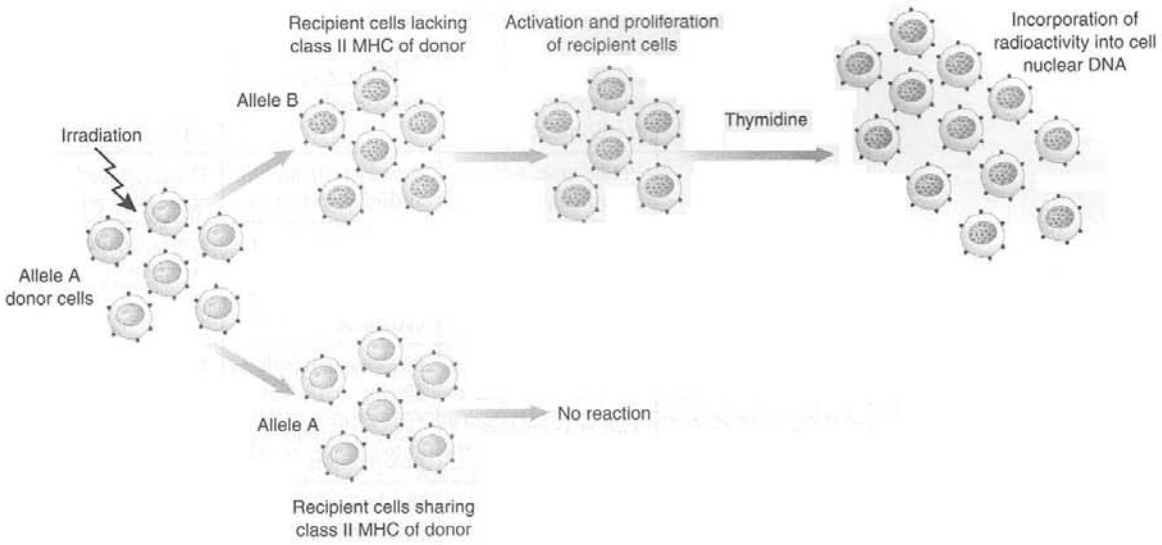

Recipient cells lacking class II MHC of donor
Activation and proliferation of recipient cells
Incorporation of radioactivity into cell nuclear DNA
Allele B
Irradiation
Thymidine
Allele A donor cells
Allele A
No reaction
Recipient cells sharing class II MHC of donor

Figure II-14-4. The One-Way Mixed Lymphocyte Response

Patients awaiting organ transplants are screened for the presence of **preformed antibodies** reactive with allogeneic HLA molecules. These can arise because of previous pregnancies, transfusions, or transplantations and can mediate hyperacute graft rejection if they exist. This test is performed as a microcytotoxicity test (as above) with patient sera tested against batteries of donor cells reflective of the potential donor pool. If a potential donor is identified, then **crossmatching** is performed, testing serum from the recipient against the lymphocytes of the potential donor.

Prevention and Treatment of Allograft Rejection

Allogeneic and even syngeneic transplantation require some degree of **immunosuppression** for the transplant to survive. Most of the treatments currently in use suffer from lack of specificity: They result in generalized immunosuppression, which leaves the host susceptible to increased risk of infection.

In A Nutshell

Immunosuppression

- Necessary for all except autografts
- Inhibits T-cell mitosis
- Inhibits T-cell function
- Kills T cells
- Blocks cytokines or costimulatory molecules

Table II-14-2. Therapies to Prevent Graft Rejection

Category of Agent	Drug	Action	Use
Mitotic inhibitors	Cyclophosphamide, methotrexate	Block proliferation	Prevent acute graft rejection
Corticosteroids	Prednisone, dexamethasone	Broad spectrum antiinflammatories	During episodes of acute graft rejection
Fungal metabolites	Cyclosporin A	Block proliferation of TH cells and production of their cytokines	Prevent acute graft rejection
Experimental monoclonals	Anti-CD3	Binds to and depletes T cells	Prevent acute graft rejection
	Anti-IL2 receptor	Inhibits T-cell proliferation	
	Anti-CD40 ligand	Blocks T-cell proliferation	

Chapter Summary

- In transplantation, tissues are taken from a donor and given to a host or recipient.
- Autografts are grafts transplanted from one location to another in the same individual.
- Syngeneic grafts are grafts transplanted between monozygotic twins.
- Allogeneic grafts are grafts transplanted between nonidentical members of the same species.
- Xenogeneic grafts are grafts transplanted from one species to another.
- During graft rejection, MHC allele products are recognized as foreign by CTLs, macrophages, and antibodies, and the graft is destroyed.
- Graft rejection is hyperacute when preformed antidonor antibodies and complement destroy the graft in minutes to hours.
- Graft rejection is accelerated when sensitized T cells are reactivated to destroy the graft in days.
- Graft rejection is acute when T cells are activated for the first time and destroy the graft in days to weeks.
- Graft rejection is chronic when antibodies, immune complexes, or cytotoxic cells destroy the graft in months to years.
- Graft-versus-host disease occurs when mature T cells inside bone marrow transplants become activated against the MHC products of the graft recipient.
- Tissue compatibility testing involves ABO blood typing, the mixed lymphocyte reaction (for class II compatibility), and the microcytotoxicity test (for class I compatibility).
- Immunosuppression is required to ensure the survival of all grafts, except autografts.
- The goals of immunosuppression are to block cell proliferation (cyclophosphamide and methotrexate); to stop inflammation (prednisone, dexamethasone); or to block proliferation of TH cells and production of their cytokines (cyclosporin A).

Review Questions

1. A 42-year-old auto mechanic has been diagnosed with end-stage renal disease. His twin brother is HLA identical at all MHC loci and volunteers to donate a kidney to his brother. What type of graft transplant terminology is correct in this situation?

   (A) Allograft
   (B) Autograft
   (C) Heterograft
   (D) Syngeneic graft
   (E) Xenograft

2. A patient with acute myelogenous leukemia (AML) undergoes irradiation and chemotherapy for his malignancy while awaiting bone marrow transplantation from a closely matched sibling. Six months after the transplant, the immune response appears to be reconstituting itself well—until 9 months postinfusion, when symptoms of generalized rash with desquamation, jaundice, and bloody diarrhea begin to appear. A second, more closely matched bone marrow donor is sought unsuccessfully, and 10 months after the transfer, the patient dies. What is the immunologic effector mechanism most closely associated with this rejection reaction?

   (A) Activated macrophages
   (B) Antibodies and complement
   (C) CD8+ lymphocytes
   (D) LAK cells
   (E) NK cells

3. A 45-year-old welder develops a severe corneal ulcer, which requires treatment with corneal transplantation. A suitable cadaver cornea is available and is successfully engrafted. What is the appropriate postsurgical treatment for this patient?

   (A) Corticosteroids, such as prednisone, for life
   (B) Fungal metabolites, such as cyclosporin A, for life
   (C) Mitotic inhibitors, such as cyclophosphamide, for life
   (D) Monoclonal anti–IL-2 receptor for life
   (E) No treatment required

4. A child who requires a kidney transplant has been offered a kidney by both parents and three siblings. A one-way mixed lymphocyte reaction between prospective donors and recipient is performed, and the stimulation indices are shown. The stimulation index is the ratio of proliferation (measured by [^{3}H]-thymidine incorporation) of the experimental group versus the negative control group. Which of the prospective donors would be the best choice?

	Irradiated Stimulator Cells					
Responder Cells	**Recipient**	**Father**	**Mother**	**Sibling 1**	**Sibling 2**	**Sibling 3**
Recipient	1.0	4.1	2.3	1.1	8.3	8.5
Father	5.3	1.0	12.3	5.6	4.9	5.9
Mother	3.2	12.6	1.0	4.5	3.9	4.8
Sibling 1	1.6	6.5	5.5	1.0	4.4	6.0
Sibling 2	7.6	5.9	4.9	4.4	1.0	7.8
Sibling 3	9.0	5.7	4.4	7.0	8.9	1.0

(A) Father
(B) Mother
(C) Sibling 1
(D) Sibling 2
(E) Sibling 3

5. In heart–lung transplantation, where the critical illness of the transplant recipient and the inability to preserve tissues from brain-dead donors often precludes tissue typing prior to transplantation surgery, a variety of experimental immunosuppressive protocols are under study. In one such experimental protocol, patients are treated with anti-CD28 antibody Fab fragments at the time of transplantation and at monthly intervals thereafter. What would be the goal of such therapy?

(A) To destroy T cells
(B) To induce anergy to transplanted tissues
(C) To inhibit mitosis in B cells
(D) To inhibit mitosis in T cells
(E) To stop inflammation

Answers and Explanations

1. **The correct answer is D.** A syngeneic graft is performed between genetically identical individuals. In human medicine, these are performed between monozygotic twins. In reality, even these "identical" individuals are not identical because minor mutations can occur during development. These sorts of grafts still require immunosuppression for success. They are, however, the best chance for success other than autografts.

   An allograft (**choice A**) is a transplant between two members of the same species who are not genetically identical. These are the most common types of transplants used in medicine, but in this vignette, the twins are described as having identical MHC haplotypes.

   An autograft (**choice B**) is a transplant from one location in the body to another. This is the only form of transplantation that will succeed without immunosuppression.

   "Heterograft" (**choice C**) is not a word that is used in transplantation immunology.

   A xenograft (**choice E**) is a transplant that is performed across species barriers.

2. **The correct answer is C.** Graft-versus-host disease (GVHD) is primarily a manifestation of sensitization of transplanted T cells against recipient tissues. The killing of mucosal and other epithelial cells is largely mediated through recognition of MHC class I incompatibility by transferred cytotoxic cells or their precursors. However, eventually, continuous priming by the host's own tissues will elicit immune responses at the level of all the cells of the immune system.

   Activated macrophages (**choice A**) are involved in the delayed-type hypersensitivity response, but are not stimulated by MHC incompatibility, so if they become involved in pathology, it has to be secondary to TH stimulation.

   Antibodies and complement (**choice B**) are not involved in GVHD. Because bone marrow is a cellular transplant, it is the cells inside it that start the problem, not accidentally transferred antibodies or complement.

   LAK cells (**choice D**) are believed to be involved in the rejection of bone marrow transplants by the recipient (host-versus-graft), but not in GVHD.

   NK cells (**choice E**) are believed to be involved in the rejection of bone marrow transplants by the recipient (host-versus-graft), but not in GVHD.

3. **The correct answer is E.** Because the eye is an immunoprivileged site, corneal transplantation is unique amongst allogeneic transplantation techniques practiced in human medicine in that it does not require immunosuppression. Other immunoprivileged sites in the human include the uterus, the testes, the brain, and the thymus. What these sites have in common is that they do not possess lymphatic vessels. For this reason, the alloantigens of the graft are not able to sensitize the recipient's lymphocytes, and the graft has an increased likelihood of acceptance.

   Corticosteroids, such as prednisone, for life (**choice A**) are required for most types of transplantation. Corticosteroids act as broad-spectrum antiinflammatories, which are particularly important in treatment of episodes of acute graft rejection.

   Fungal metabolites, such as cyclosporin A, for life (**choice B**) would be necessary for most types of transplantation. These agents act by blocking proliferation of TH cells and production of their cytokines.

   Mitotic inhibitors, such as cyclophosphamide, for life (**choice C**) would be necessary for most types of transplantation. These agents act by blocking proliferation of cells.

   Monoclonal anti–IL-2 receptor for life (**choice D**) is an experimental protocol that would inhibit T-cell proliferation. It would not be necessary in the case of corneal transplantation.

4. **The correct answer is C.** The lowest stimulation index (and the lowest amount of proliferation) is shown between sibling 1 and the prospective recipient, both when the donor cells are used as stimulators and as responders. This means (most importantly) that the recipient will make little response to the graft and (less importantly, except in graft-versus-host disease) that the donor will make little response against the recipient.

   The father (**choice A**) is not the best choice of donors because the recipient makes four times the proliferative response to his cells as to those of sibling 1.

   The mother (**choice B**) is not the best choice of donors because the recipient makes twice the proliferative response to her cells as to those of sibling 1. She would be the second-best choice, unless sibling 1 had an incompatible ABO blood group.

   Sibling 2 (**choice D**) is not the best choice of donors because the recipient makes eight times the proliferative response to his cells as to those of sibling 1.

   Sibling 3 (**choice E**) is not the best choice of donors because the recipient makes eight times the proliferative response to his cells as to those of sibling 1.

5. **The correct answer is B.** If a patient were treated with the Fab portions of antibodies to the CD28 molecule, this would block the binding of CD28 (on T cells) to B7 on antigen-presenting cells. Because this costimulatory signal is necessary as a second signal following TCR binding, the cell receives no second signal and becomes unresponsive (anergic) to that antigen.

   To destroy T cells (**choice A**) is the goal of treatment with experimental monoclonals such as anti-CD3 antibodies. These bind to and deplete T cells, but act in a nonspecific fashion, so there is increased susceptibility to infection.

   To inhibit mitosis in B cells (**choice C**) is not a goal of any of the therapies against graft rejection. T cells are at the root of all types of graft rejection, with the possible exception of hyperacute rejection based on ABO blood group incompatibilities.

   To inhibit mitosis in T cells (**choice D**) is the goal of agents such as cyclophosphamide and methotrexate.

   To stop inflammation (**choice E**) is the goal of corticosteroids such as prednisone and dexamethasone. These are broad-spectrum antiinflammatories used during periods of acute graft rejection.

Cancer Immunology 15

What the USMLE Requires You to Know

- The immunologic mechanisms of surveillance against tumors
- The major mechanisms of tumor immune evasion
- The rationales of major tumor immunotherapy techniques

Tumors express antigens that are recognized by the immune system, but most tumors are weakly immunogenic, and immune responses frequently fail to stop their growth. These antigens may be:

- Products of oncogenes (Ras) or tumor suppressor genes (p53)
- Mutants of cellular genes not involved in tumorigenesis
- Products of genes that are silent in most normal tissues
- Products of overexpressed genes
- Products of oncogenic viruses (E6 and E7 proteins of HPV)
- Oncofetal antigens (α-fetoprotein)
- Differentiation antigens normally present in the tissue of origin (e.g., prostate-specific antigen)

In A Nutshell

Tumors are weakly immunogenic.

The recognition of tumor antigens by CTLs is the major effector mechanism in antitumor immunity, but other effectors such as antibody, macrophages, and NK cells may be important in some cases. Cytotoxic CD8+ cells perform a surveillance function by recognizing tumor peptides presented in the groove of the class I MHC molecule. If tumor-specific peptides are presented by APCs that have ingested them, then costimulatory molecules are engaged, and the response becomes more powerful. CD4+ TH cells probably also play a role by providing cytokines that stimulate CTL development and cytokines such as TNF and IFN-γ, which upregulate expression of MHC molecules on the surface of tumor cell targets.

In A Nutshell

Antitumor Effector Mechanisms

- CTLs
- Antibody (with complement or ADCC)
- Macrophages
- NK cells

Antibodies may kill tumor cells by activating complement or assisting ADCC, but this arm of the immune response is clearly secondary to the generation of tumor-specific CTLs.

In cases where tumor cells have reduced expression of class I MHC molecules, NK cells may play a major protective role. Macrophages activated by IFN-γ from TH1 cells have increased tumoricidal capacity and produce TNF, which has been shown to induce thrombosis in tumor blood vessels.

In A Nutshell

Tumor Immune Evasion

- Decreased MHC expression
- Antigen-loss variants
- TGF-β inhibits effector functions
- FasL induces apoptosis
- Recognition of antigen by TCR without costimulatory signal induces tolerance
- Antigens masked by mucopolysaccharides

In A Nutshell

Tumor Immunotherapy

- Vaccination
- Increase costimulatory molecules
- Stimulate T cells
- Antitumor Abs
- Immunotoxins
- Expand cytotoxic cells in culture

Clinical Correlate

The hepatitis B vaccine was technically the first antitumor vaccine to be placed on the market.

Immune Evasion by Tumors

Several mechanisms exist by which tumors seem to evade the development or function of anti-tumor immune responses.

- Class I MHC expression may be downregulated.
- Antigen-loss variants emerge in rapidly growing tumors.
- Tumors may not stimulate CTLs if they lack costimulatory molecules (causing tolerance).
- Transforming growth factor-β secreted by some tumors inhibits proliferation and effector functions in lymphocytes and macrophages.
- Fas ligand expressed on some tumors may induce apoptotic death of lymphocytes.
- The antigens of tumors may be masked by sialic acid–containing mucopolysaccharides.

Immunotherapy for Tumors

Immunotherapy for tumors is designed to augment development of an active immune response against the tumor or passively deliver tumor-specific immune effectors to patients:

- Vaccination with tumor cells or antigens
- Administration of tumor cells modified to express high levels of costimulators
- Administration of cytokines that stimulate T-cell proliferation and differentiation
- Administration of antitumor antibodies
- Administration of antibodies conjugated to toxic drugs (immunotoxins)
- Administration of tumor-reactive T cells and NK cells expanded in culture by growth factors (LAK cells)

Clinical Correlate

The Therapeutic Use of Interferons

Since the first description of interferons almost 50 years ago, a multitude of dramatic immunomodulatory roles have been discovered for this group of proteins. As a group, interferons induce increases in the expression of class I and II MHC molecules and augment NK cell activity. They increase the efficiency of presentation of antigens to both cytotoxic and helper cell populations. Cloning of the genes that encode α, β, and γ interferons has made it possible to produce amounts of these products, which make their use clinically practical.

Interferon has well-known antiviral activity and has been used in the treatment of hepatitis B and C infections. Within cancer therapy, IFN-α has shown promise in treatment of hairy B-cell leukemia, chronic myelogenous leukemia, and Kaposi sarcoma.

Interferon-β was the first drug shown to have a positive effect on young adults with multiple sclerosis. Patients treated with IFN-β enjoy longer periods of remission and reduced severity of relapses.

Interferon-γ is being used in the treatment of chronic granulomatous disease (CGD). This molecule is a potent inducer of macrophage activation and a promoter of inflammatory responses. Its application appears to significantly reverse the CGD patient's inability to generate toxic oxygen metabolites inside phagocytic cells.

The side effects of IFN therapy are fortunately mild and can be managed with acetaminophen. They include headache, fever, chills, and fatigue and diminish with continued treatment.

Chapter Summary

- Most tumors express antigens that are weakly immunogenic.
- CTLs are the major antitumor effector cell.
- Antibodies can act with complement or assist in ADCC to kill tumor cells.
- Macrophages activated by TH1-cell–produced IFN-γ and NK cells can kill tumor cells in some cases.
- TNF and IFN-γ from TH1 cells increase expression of MHC class I on tumor cells, making them more susceptible to CTL killing.
- Tumors evade the immune response by downregulating MHC expression, lacking costimulatory molecules (and thereby inducing tolerance), inhibiting immune cell proliferation, inducing apoptosis of immune cells, and masking antigens in mucopolysaccharides.

Review Questions

1. A patient with advanced metastatic melanoma decides to join an experimental treatment protocol in the hope that it will cause regression of his tumor masses. Malignant cells are aspirated from several of his lesions and transfected in vitro with the gene encoding GM-CSF production. The transfected tumor cells are then reinfused into the patient. Mobilization of which of the following cells from the bone marrow would be likely to result from this treatment?

   (A) Antigen-presenting cells
   (B) B lymphocytes
   (C) NK cells
   (D) Plasma cells
   (E) T lymphocytes

2. A patient with a B-cell lymphoma is referred to an oncology clinic for the analysis of his condition. The malignant cells are found to be producing IgM monomers. Which of the following therapeutic regimens is most likely to destroy the malignant cells and no others?

   (A) Anti-CD3 antibodies plus complement
   (B) Anti-CD19 antibodies plus complement
   (C) Anti-CD20 antibodies plus complement
   (D) Anti-idiotype antibodies plus complement
   (E) Anti-μ chain antibodies plus complement

3. An experimental protocol for the protection of high-risk populations against human papilloma virus and cervical intraepithelial neoplasia involves the production of a recombinant DNA vaccine. The genes of the virus that encode the E6 and E7 proteins are inserted into a viral vector and inoculated intramuscularly into the patient. Which of the following would be the goal of such treatment?

   (A) To inactivate viral oncogenes
   (B) To increase MHC class I expression on tumor cells
   (C) To produce anti-idiotype antibodies
   (D) To protect natural cellular antioncogenes
   (E) To stimulate CD8+ T lymphocytes

4. A woman with advanced metastatic breast cancer undergoes a radical mastectomy, followed by irradiation and chemotherapy. After a 2-year remission, a metastatic focus appears, and she enrolls in an experimental treatment protocol. In it, a sample of her aspirated bone marrow is treated with GM-CSF, TNF-α, and IL-2, then pulsed with membrane fragments of her tumor cells and reinfused. Which of the following cell subpopulations is the most directly targeted by this treatment?

   (A) B lymphocytes
   (B) Cytotoxic T lymphocytes
   (C) NK cells
   (D) TH1 cells
   (E) TH2 cells

5. A 6-year-old child from Zimbabwe is admitted to a U.S. oncology center for treatment of an advanced case of Burkitt lymphoma. Analysis of the malignant cells reveals that they are lacking MHC class I antigens on their surface. Which of the following cytokines produced by recombinant DNA technology could be injected into his solid tumor to increase this tumor cell's susceptibility to CD8+-mediated killing?

   (A) IFN-γ
   (B) IL-1
   (C) IL-2
   (D) IL-10
   (E) TNF-α

Answers and Explanations

1. **The correct answer is A.** Tumor cells transfected with the gene encoding GM-CSF would produce granulocyte/monocyte colony-stimulating factor. This is a cytokine that acts on the bone marrow to cause production and mobilization of antigen-presenting cells. The goal of such therapy would be to induce the production of antigen-presenting cells, which might increase the presentation of tumor-cell antigens to cells important in cell-mediated cytotoxicity.

   B lymphocytes (**choice B**) would not be mobilized by such a treatment. The cytokine that favors development of lymphoid precursors in the bone marrow is IL-7.

   NK cells (**choice C**) would not be mobilized by such a treatment. Although NK cells are granular, they are derived from lymphoid, not granulocyte/monocyte, precursors. The cytokine that favors development of lymphoid precursors in the bone marrow is IL-7.

   Plasma cells (**choice D**) are produced in the secondary lymphoid organs and submucosa. IL-7, which stimulates lymphoid precursors in the bone marrow, would have an indirect effect on plasma cell production, but they are not mobilized from the bone marrow.

   T lymphocytes (**choice E**) would not be mobilized by such a treatment. The cytokine that favors development of lymphoid precursors in the bone marrow is IL-7.

2. **The correct answer is D.** Because malignant cells are clonal in origin, all the cells in this patient's lymphoma should be producing IgM monomers of a single idiotype. Treatment with anti-idiotype antibodies plus complement, therefore, would specifically kill only malignant cells, and leave all other B lymphocytes unharmed.

   Anti-CD3 antibodies plus complement (**choice A**) would kill all T lymphocytes in the body. This lymphoma is clearly of B-cell origin because it is bearing IgM monomers.

   Anti-CD19 antibodies plus complement (**choice B**) would kill all B lymphocytes in the body. It would not specifically target malignant cells.

   Anti-CD20 antibodies plus complement (**choice C**) would kill all B lymphocytes in the body. It would not specifically target malignant cells.

   Anti-μ chain antibodies plus complement (**choice E**) would kill all mature and naive B cells and immature B cells that had completed VDJ rearrangement of their heavy chain genes. It would not be specific for malignant cells.

3. **The correct answer is D.** In human papilloma virus infections, production of viral proteins E6 and E7 inactivates two cellular tumor suppressor genes, p53 and p110. The vaccine in this case is designed to create an immune response against these viral proteins, and in so doing, protect the function of two normal cellular antioncogenes.

   To inactivate viral oncogenes (**choice A**) is not correct because proteins E6 and E7 inactivate cellular antioncogenes.

   To increase MHC class I expression on tumor cells (**choice B**) is not correct because proteins E6 and E7 do not affect MHC class I expression. IFN treatment has been shown to increase expression of MHC class I products on cells.

   To produce anti-idiotype antibodies (**choice C**) is not the goal of this vaccine because anti-idiotype antibodies can only be produced by repeated inoculations of monoclonal antibodies. This vaccine is inoculating E6 and E7 viral proteins, not antibodies.

   To stimulate CD8+ T lymphocytes (**choice E**) would be an indirect benefit of this vaccine because it should generate cells capable of responding to the viral E6 and E7 proteins. Because the E6 and E7 proteins combine with and inactivate cellular anti-oncogenes, however, CTLs alone would not be sufficient to protect against malignant transformation.

4. **The correct answer is D.** The goal of this therapy is to provide an increased population of activated antigen-presenting cells primed with tumor cell antigens so that these can be presented to the TH cells involved in stimulation of cell-mediated immunity. The TH1 cell is the first cell listed which would be activated by such a treatment.

   B lymphocytes (**choice A**) would not be stimulated by such treatment. B lymphocytes bind to and are activated by unprocessed (not cell-bound) antigens.

   Cytotoxic T lymphocytes (**choice B**) would be indirectly stimulated by this treatment. Cytokines secreted by the activated TH1 cells would have the effect of increasing the number and cytotoxic activity of killer cells.

   NK cells (**choice C**) would be indirectly stimulated by this treatment. Cytokines secreted by the activated TH1 cells would have the effect of increasing the number and cytotoxic activity of killer cells.

   TH2 cells (**choice E**) would be stimulated by this treatment, but this is not the major goal of such therapy. TH2 cells stimulate humoral immunity, which is not the most important protective mechanism against tumor cells.

5. **The correct answer is A.** IFNs of all types increase cellular expression of MHC class I and II products. Because CD8+ cells recognize their targets by MHC class I–dependent mechanisms, increases in the amount of these antigens on tumor-cell targets would increase susceptibility to cytotoxic killing.

   IL-1 (**choice B**) does not increase MHC class I molecule expression. The endogenous pyrogen is responsible for alteration of the hypothalamic temperature set point during acute inflammatory events.

   IL-2 (**choice C**) does not increase MHC class I molecule expression. It is produced by TH cells and causes proliferation of many classes of lymphocytes.

   IL-10 (**choice D**) does not increase MHC class I molecule expression. It is a product of TH2 cells and inhibits TH1 cells; thus, it inhibits the cell-mediated arm of the immune response.

   TNF-α (**choice E**) does not increase MHC class I molecule expression. It may act directly on tumor cells to cause their necrosis and decrease angiogenesis. It is a product of TH1 cells that stimulates the effector cells of cell-mediated immunity.

Laboratory Techniques in Immunology

16

What the USMLE Requires You to Know

- The procedures for quantification of antigen–antibody complexes
- The general procedures and applications of agglutination, fluorescent antibody, radioimmunoassay, ELISA, Western blot, and flow cytometry
- How to interpret data from these tests to diagnose immunologic or microbial diseases

Many diagnoses in infectious disease and pathology would not be possible without laboratory procedures that identify antibodies or antigens in the patient. This chapter will illustrate the most commonly used techniques.

Antigen–Antibody Interactions

Interaction of antigen and antibody occurs in vivo, and in clinical settings it provides the basis for all immunologically based tests. The formation of immune complexes produces a visible reaction that is the basis of precipitation and agglutination tests. Agglutination and precipitation are maximized when multiple antibody molecules share the binding of multiple antigenic determinants, a condition known as **equivalence**. In vivo, the precipitation of such complexes from the blood is critical to the trapping of invaders and the initiation of the immune response in the secondary lymphoid organs, as well as the initiation of the pathologic phase of many immune complex–mediated diseases. In vitro, the kinetics of such reactions can be observed by titration of antigen against its specific antibody.

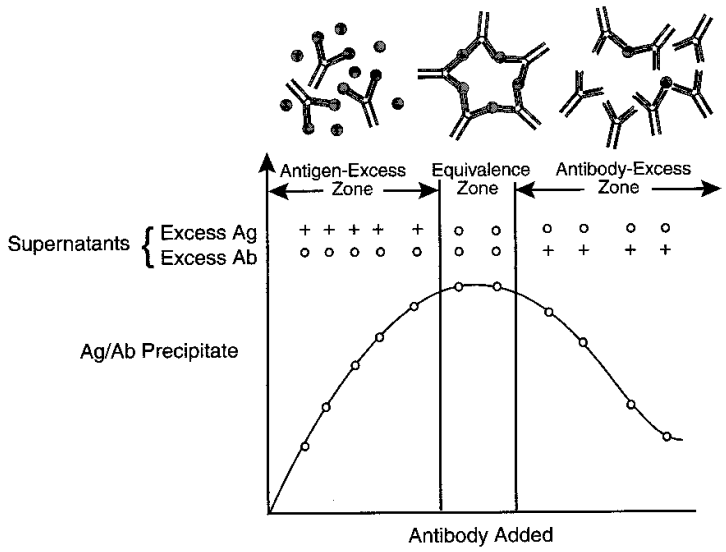


Figure II-16-1. Precipitation of Ag–Ab Complexes During Titration of Ag with Ab

In A Nutshell

- Ag excess–early in infection
- Equivalence–all available Ag complexed with Ab = window period
- Ab excess–late in infection

Figure II-16-1 demonstrates the normal progression of the antibody response during many infectious diseases. At the start of the infection, the patient is in a state of **antigen excess** because the pathogen is proliferating in the host and the development of specific antibodies has not yet begun. As the patient begins to make an adequate antibody response, he enters the **equivalence zone**, when all available antigen is complexed with antibody, and neither free antigen nor free antibody can be detected in the serum. Finally, as the infection is resolved, the patient enters the **antibody excess** zone, when more antibody is being produced than is necessary to precipitate all available antigen. The clinical demonstration of this phenomenon is most elegantly seen in our use of the serologic diagnosis of hepatitis B infection. Early in the course of this infection, HBsAg and HBeAg are easily detectable in the blood. The patient is in the antigen excess zone. As the patient enters the **window period** (the equivalence zone), the antigens disappear from the circulation because they are being removed by antibody precipitation. The respective antibody titers (HBsAb and HBeAb) rise in the serum as the patient enters the antibody excess zone and resolves the infection. Although the "window period" in the hepatitis B infection is used exclusively to connote the absence of HBsAg and HBsAb from the serum (because it is the only antigen–antibody response that has a clinical significance in the prognosis of disease), an equivalence zone, or "window period," is a universal stage in the development of any antibody–antigen interaction.

Agglutination

Agglutination tests are widespread in clinical medicine and are simply a variation on precipitation reactions in which the antigen is a particle and not a soluble material. The two particles most commonly used in medicine for this purpose are RBCs and latex beads, and both will settle out of suspension in the form of a carpet of antibody-bound particles in the presence of appropriate antibodies. Latex bead agglutination tests are available for the diagnosis of cerebrospinal infections such as *Haemophilus*, pneumococcus, meningococcus, and *Cryptococcus neoformans*. In each of these cases, antibodies against these organisms are conjugated to latex beads, and the presence of pathogen antigens in the CSF is detected by the subsequent agglutination of those

beads. RBC agglutination reactions are important in defining ABO blood groups (*see* Chapter 14), diagnosing Epstein-Barr virus infection (the monospot test), and the Coombs test for Rh incompatibility.

Two variations of the Coombs test exist. The direct Coombs is designed to identify maternal anti-Rh antibodies already bound to infant RBCs or antibodies bound to RBCs in patients with autoimmune hemolytic anemia. The indirect Coombs test is used to identify Rh-negative mothers who are producing anti-Rh antibodies of the IgG isotype, which may be transferred across the placenta to harm Rh-positive fetuses.

In A Nutshell

Direct Coombs–detect antibodies bound to RBC

Indirect Coombs–detect production of anti-RBC antibodies

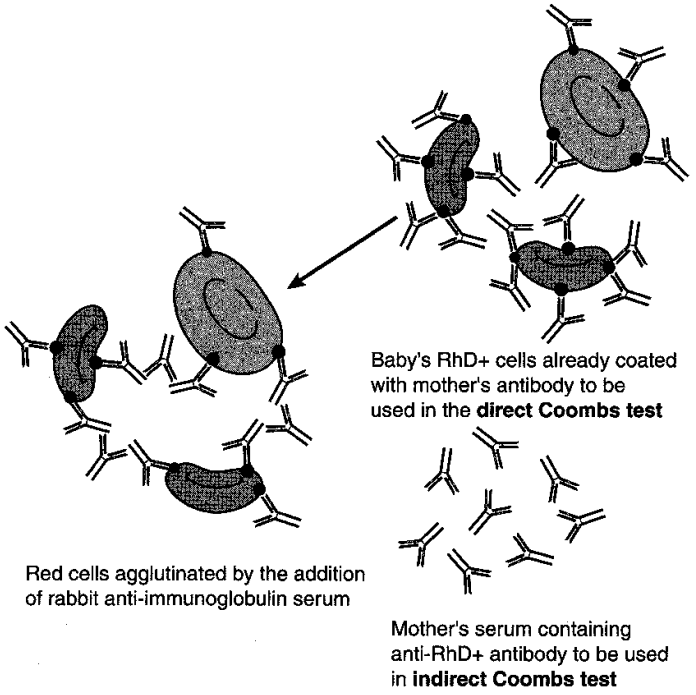

Baby's RhD+ cells already coated with mother's antibody to be used in the **direct Coombs test**
Red cells agglutinated by the addition of rabbit anti-immunoglobulin serum
Mother's serum containing anti-RhD+ antibody to be used in **indirect Coombs test**

Figure II-16-2. Coombs Test

Fluorescent Antibody Tests

The direct fluorescent antibody test (DFA) is used to detect and localize antigen in the patient. The tissue sample to be tested is treated with antibodies against that particular antigen that have been labeled with a fluorescent dye. If the antigen is present in the tissues, the fluorescent-labeled antibodies will bind, and their binding can be detected with a fluorescence microscope. Variations of this test are used to diagnose respiratory syncytial virus, herpes simplex-1 and -2, and *Pneumocystis* infections.

In A Nutshell

DFA identifies Ag in tissues.

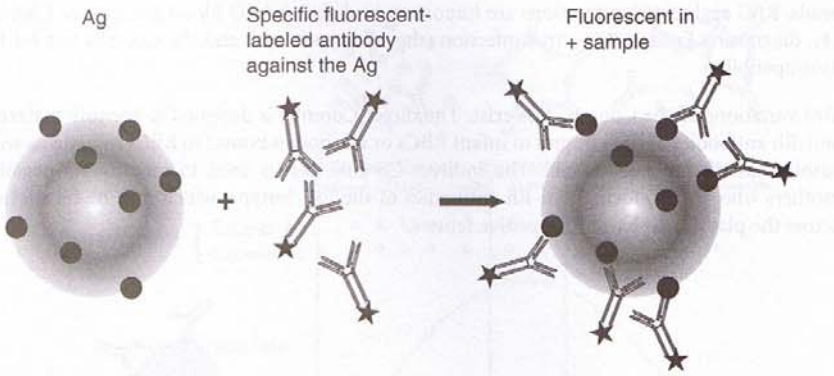


Figure II-16-3. DFA Test

In A Nutshell

IFA detects Abs in the patient.

The **indirect fluorescent antibody test (IFA)** is used to detect pathogen-specific antibodies in the patient. In this case, a laboratory-generated sample of infected tissue is mixed with serum from the patient. A fluorescent dye–labeled anti-immunoglobulin (raised in animals) is then added. If binding of antibodies from the patient to the tissue sample occurs, then the fluorescent antibodies can be bound, and fluorescence can be detected in the tissue by microscopy. This technique is used to detect antinuclear antibodies, anti-ds DNA antibodies, antithyroid antibodies, antiglomerular basement-membrane antibodies, and anti–Epstein-Barr virus viral-capsid antigen antibodies.

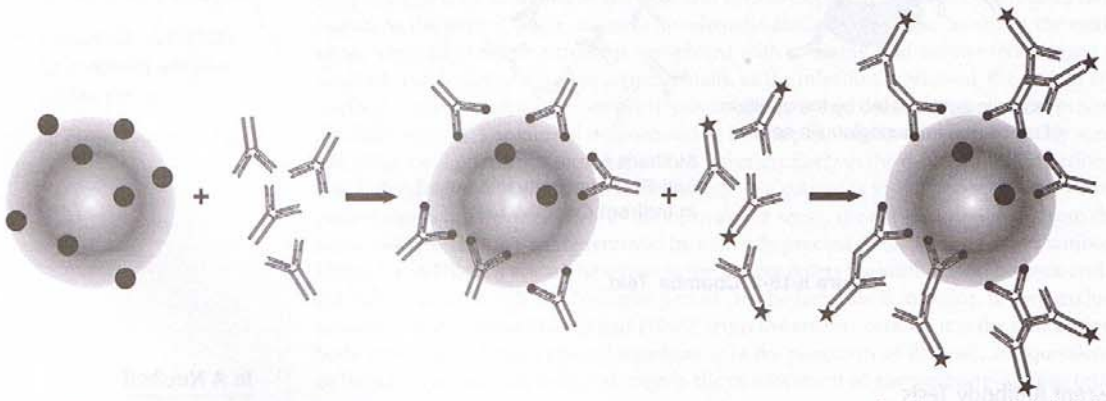

Test Ag
Ab (human immunoglobulin) from patient
Anti-human γ-globulin labeled with fluorescent dye
If the test Ag is fluorescent following these steps, then the patient had antibody against this antigen in their serum.

Figure II-16-4. Indirect FA Test

Radioimmunoassay (RIA) and Enzyme-Linked Immunoabsorbent Assay (EIA or ELISA)

RIA and ELISA are extremely sensitive tests (as little as 10^{-9} g of material can be detected) that are common in medical laboratories. They can be used to detect the presence of hormones, drugs, antibiotics, serum proteins, infectious disease antigens, and tumor markers. Both tests are conducted similarly, but the RIA uses the detection of a radiolabeled product and the ELISA detects the presence of enzyme-mediated color changes in a chromogenic substrate.

In the screening test for HIV infection, the ELISA is used, with the p24 capsid antigen from the virus coated onto microtiter plates. The serum from the patient is then added, followed by addition of an enzyme-labeled antihuman immunoglobulin. Finally, the enzyme substrate is added, and the production of a color change in the well can be observed if all reagents bind one another in sequence.

In A Nutshell

ELISA and RIA

- Extremely sensitive
- ELISA is screening test for HIV

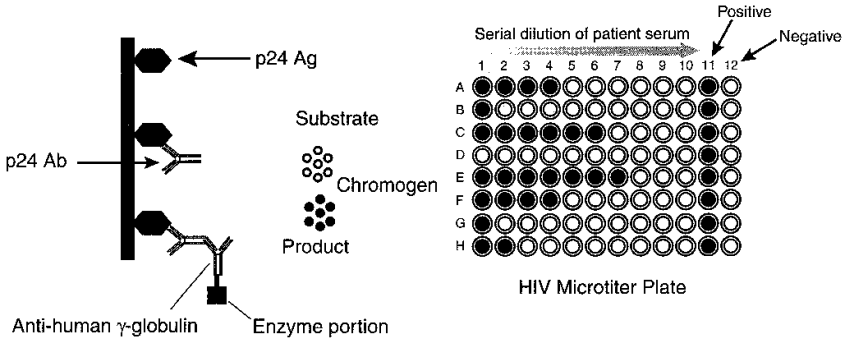


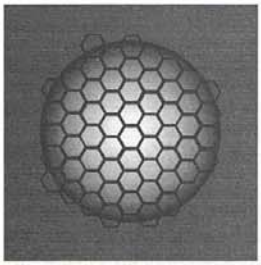
Figure II-16-5. ELISA Test

Western Blot or Immunoblot

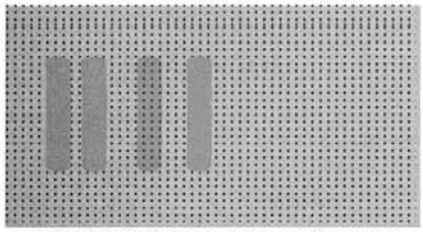
The Western blot is primarily used in medicine for confirmation of HIV infection in a patient who has seroconverted and is thus positive by the ELISA test. Because the ELISA suffers from the detection of some false-positives, this follow-up step is essential for the diagnosis of HIV infection. In this test, the antigens of the virus are separated in an electric field and blotted onto nitrocellulose paper. The serum of the patient is then added and allowed to bind to any antigens that it recognizes. Next, antihuman immunoglobulin antibodies conjugated to either enzyme or radioactive labels are added, to bind to the previously bound patient's antibodies (if any). The result of color change in the case of an enzyme system or radioactivity in the case of radiolabeling can then be visualized. An HIV Western blot is judged to be "reactive" if the patient has reacted to at least two of three gene products (p24, gp41, gp120).

In A Nutshell

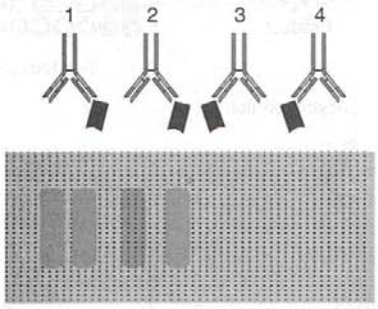
Western blot is a confirmatory test for HIV.



Viral organism separated into constituent proteins



Proteins separated in SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis)



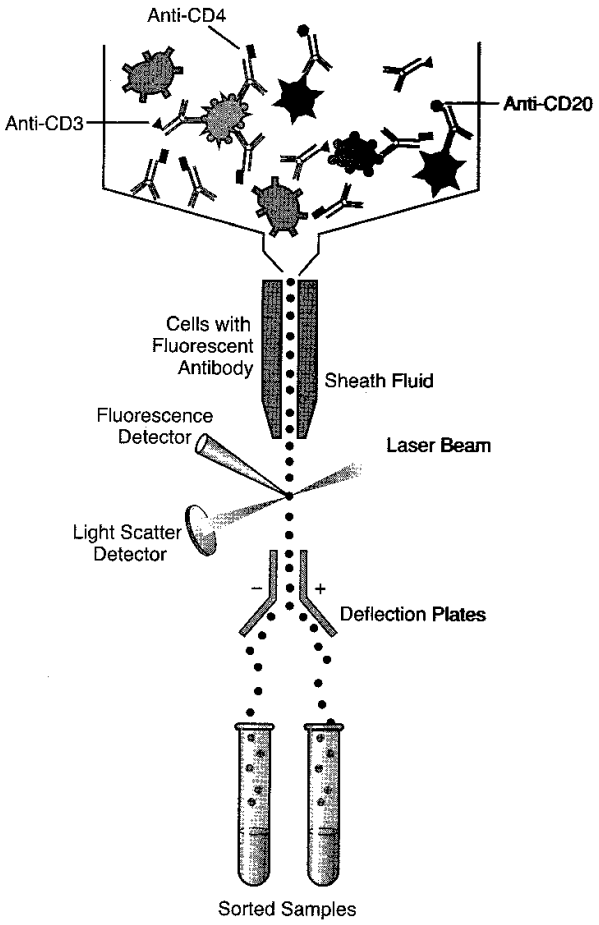
Separated proteins are transferred to nitrocellulose sheet and reacted with patient's serum. Next, antihuman γ-globulin labeled with an enzyme is reacted for color development and identification. Four different antibodies were identified from this patient.

Figure II-16-6. Western Blot Test

In A Nutshell

Flow cytometry analyzes cell populations in a complex mixture.

This procedure is used to rapidly analyze cell types in a complex mixture and sort them into different populations based on their binding to specific fluorescent dyes. By using antibodies against specific cell-surface markers conjugated to different fluorescent dyes, it is possible to analyze the relative numbers of cells present in a specific tissue location. The apparatus then generates a computerized histogram, reflecting the intensity of the fluorescence and the numbers of cells that have borne that marker. The histogram is produced as a scatter diagram, with cells expressing increasing fluorescence with one dye plotted along the *x*-axis, and increasing fluorescence with the other dye along the *y*-axis. Cells with high fluorescence from both dyes will therefore be found in the top right quadrant.



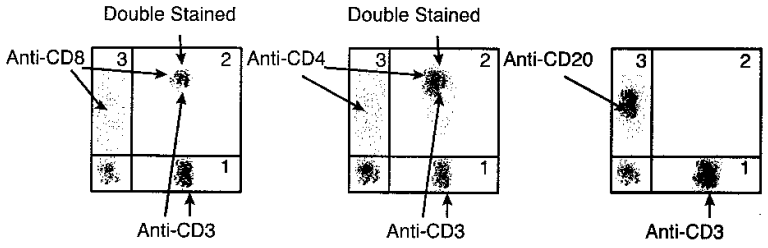


Figure II-16-7. Flow Cytometric Analysis

Chapter Summary

- Antigen–antibody interactions can be visualized in vitro and serve as the basis of many medical diagnostic tests.
- Early in infection when antigen is in excess, only the pathogen's antigens can be detected in patient serum. As antibodies begin to be produced, complexes are formed that precipitate out of the circulation, and the patient enters the equivalence zone. Rising titers of antibody are measured as the patient progresses into the antibody excess zone.
- Agglutination tests are used to measure antibodies that can cause clumping of particles (RBCs and latex beads).
- The direct Coombs test is an agglutination test that detects infants at risk for developing erythroblastosis fetalis. The indirect Coombs test is used to diagnose the presence of antibody in mothers who are at risk of causing this condition in their children.
- The direct fluorescent antibody test is used to detect and localize antigen in patient tissues. The indirect fluorescent antibody test is used to detect antibody production in a patient.
- The radioimmunoassay and enzyme-linked immunoabsorbent assay are extremely sensitive tests that can be modified to detect antigens or antibodies. The ELISA is used as a screening test for HIV infection.
- The Western blot is the confirmatory test for HIV infection.
- Flow cytometry is used to analyze and separate cell types out of complex mixtures.

Review Questions

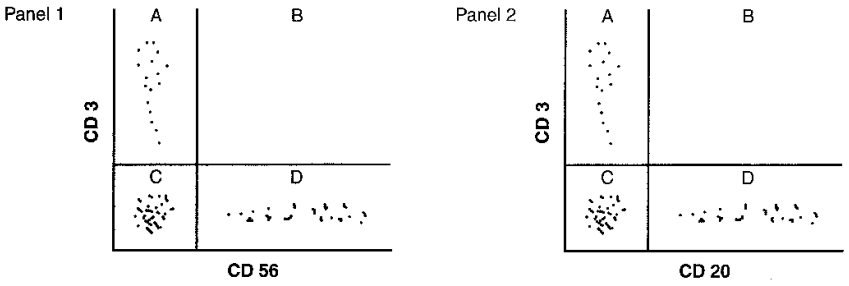
1. A 16-year-old runaway heroin user visits a family planning/STD clinic irregularly to receive birth control pills. In April 2004, the standard HIV screen performed by this clinic reports back that her test was positive. What does the primary test for HIV infection use?

   (A) Electrophoresis of HIV antigens in polyacrylamide gel
   (B) HIV antigen covalently coupled to RBC, patient serum, and anti-immunoglobulin
   (C) HIV antigen covalently coupled to RBC, patient serum, and complement
   (D) HIV antigen, patient serum, anti-immunoglobulin serum, and enzyme-substrate ligand
   (E) HIV antigen, patient serum, anti-immunoglobulin serum, and radioactive ligand

2. A direct Coombs test was performed on a baby in its seventh month of gestation. The mother has had trouble with two earlier pregnancies, and she has never received RhoGAM™. The physician is concerned about the possibility of erythroblastosis fetalis. What ingredients would be necessary to perform this procedure?

   (A) Mother's serum plus RhoGAM plus Coombs reagent
   (B) Mother's serum plus Rh-RBCs plus Coombs reagent
   (C) RhoGAM plus Rh+ RBCs from the baby
   (D) Rh+ RBCs from the baby plus Coombs reagent
   (E) Rh+ RBCs plus mother's serum plus Coombs reagent

3. A patient with Chediak-Higashi syndrome is analyzed for ability to mobilize NK cells into the peripheral blood. His peripheral blood leukocytes are treated with fluorescent-labeled antibodies to CD3, CD56, and CD20 before they are passed through a fluorescence-activated cell sorter. The computer-generated results of this process are shown. In which quadrant of which panel would the natural killer cells be found?



   (A) Panel 1, quadrant A
   (B) Panel 1, quadrant B
   (C) Panel 1, quadrant C
   (D) Panel 1, quadrant D
   (E) Panel 2, quadrant A
   (F) Panel 2, quadrant B
   (G) Panel 2, quadrant C
   (H) Panel 2, quadrant D

4. In both ABO blood typing and the Coombs test for detection of hemolytic disease of the newborn, agglutination of coated erythrocytes is a positive test result. Why is addition of Coombs reagent not a necessary step in ABO blood typing?

   (A) All antibodies made in response to blood glycoproteins are IgG
   (B) Complement-mediated lysis is not important in ABO incompatibilities
   (C) Coombs serum identifies only anti-Rh antibodies
   (D) IgM pentamers are large enough to agglutinate erythrocytes directly
   (E) The high titer of natural isohemagglutinins makes Coombs reagent unnecessary

5. A young woman is in the care of an infertility specialist for evaluation of her inability to conceive since her marriage 5 years ago. As a first step in her examination, cervical scrapings are tested for the possibility of undiagnosed infection with *Chlamydia trachomatis*, which could cause fallopian tube scarring. Which of the following diagnostic tests could be used to identify chlamydial antigens in this specimen?

   (A) Direct fluorescent antibody test
   (B) Enzyme-linked immunosorbent assay (ELISA)
   (C) Indirect fluorescent antibody test
   (D) Radioimmunoassay
   (E) Western blot

Answers and Explanations

1. **The correct answer is D.** The standard screening test for HIV infection is the enzyme-linked immunosorbent assay, or ELISA. In this test, the virus p24 antigen is coated onto microtiter plates. Serum from the test subjects is added, followed by antihuman-immunoglobulin, which is labeled with an enzyme. When the substrate for the enzyme is added, if the antibodies listed have bound in sequence, there will be a color change in that microtiter well.

   Electrophoresis of HIV antigens in polyacrylamide gel (**choice A**) describes the Western blot, which is used as a confirmatory test of HIV infection.

   HIV antigen covalently coupled to RBC, patient serum, and anti-immunoglobulin (**choice B**) describes an erythrocyte agglutination test. There is no such test in use for diagnosis of HIV. The indirect Coombs test, which is used to detect Rh– mothers who have become sensitized to the Rh antigens of their fetuses, operates on this principle, however.

   HIV antigen covalently coupled to RBC, patient serum, and complement (**choice C**) describes either a complement-fixation or complement-mediated hemolysis assay. There is no such test in use for the diagnosis of HIV.

   HIV antigen, patient serum, anti-immunoglobulin serum, and radioactive ligand (**choice E**) describes a radioimmunoassay. This is not used in the standard screening for HIV.

2. **The correct answer is D.** If the child is developing hemolytic disease of the newborn, then his erythrocytes will already be coated with maternal anti-Rh antibodies. Adding Coombs serum (antihuman gammaglobulin) to the baby's RBCs then will cause agglutination. This is the direct Coombs test.

   Mother's serum plus RhoGAM plus Coombs reagent (**choice A**) is not a set of reagents that will accomplish any diagnosis. RhoGAM is anti-RhD immunoglobulin, which is given to Rh– mothers at the termination of any Rh+ pregnancy. If the mother is sensitized, she is making IgG antibodies of the same specificity. Adding these three reagents together would tell you nothing of the baby's condition.

   Mother's serum plus Rh-RBCs plus Coombs reagent (**choice B**) is not a set of reagents that will accomplish any diagnosis. If the mother is Rh–, she will not make a response to Rh– RBCs, and addition of Coombs reagent will accomplish nothing.

   RhoGAM plus Rh+ RBCs from the baby (**choice C**) is not a set of reagents that will accomplish any diagnosis. RhoGAM will bind to Rh+ RBCs from the baby by definition, but adding these reagents together would tell you nothing of the baby's condition.

   Rh+ RBCs plus mother's serum plus Coombs (**choice E**) is the set of reagents necessary for the performance of the indirect Coombs test. This is a test used to determine if the mother is making IgG anti-Rh antibodies, which could cross the placenta and harm a fetus. The question asks about the direct Coombs test, not the indirect.

3. **The correct answer is D.** The cell surface marker that would be useful to identify NK cells is CD56. The cells that have the highest fluorescence with antibodies to CD56 are found in quadrant D of panel 1.

   Panel 1, quadrant A (**choice A**) contains the cells with maximum fluorescence with antibodies to CD3. These would be T lymphocytes.

   Panel 1, quadrant B (**choice B**) contains the cells double-labeled with CD3 and CD56. Because CD3 is the pan-T-cell marker and CD56 is an NK-cell marker, there are no double-labeled cells in this case.

   Panel 1, quadrant C (**choice C**) contains the cells that have background fluorescence with both CD3 and CD56. These are non-T, non-NK cells, so they could be B lymphocytes or any other leukocyte.

Panel 2, quadrant A (**choice E**) contains the cells with maximum fluorescence with antibodies to CD3. These are T lymphocytes.

Panel 2, quadrant B (**choice F**) contains double-labeled cells, which fluoresce with both antibody to CD3 and antibody to CD20. Because CD3 is a T-cell marker and CD20 is a B-cell marker, there are no cells in this quadrant.

Panel 2, quadrant C (**choice G**) contains the cells with background fluorescence with both antibodies to CD3 and CD20. These would be non-B, non-T cells and would contain some NK cells, but other leukocytes would be included here, so this is not the best choice.

Panel 2, quadrant D (**choice H**) contains the cells with maximum fluorescence with antibody to CD20, which is a B-cell marker.

4. **The correct answer is D.** Coombs reagent is antihuman IgG. It is necessary in the direct and indirect Coombs tests because in those cases, one is looking for IgG antibodies that could be transported across the placenta to harm an unborn child. IgG is a much smaller molecule than IgM, and is not capable of agglutinating erythrocytes without the addition of a "developing" antibody. In the ABO blood typing test, the important isohemagglutinins are of the IgM variety, capable of agglutinating erythrocytes by themselves because of their sheer size.

   The statement that all antibodies made in response to blood glycoproteins are IgG (**choice A**) is not true because isohemagglutinins against ABO blood group antigens are IgM.

   The statement that complement-mediated lysis is not important in ABO incompatibilities (**choice B**) is not true because isohemagglutinins of the IgM variety are extremely powerful activators of complement-mediated lysis. The agglutination tests here, however, do not use complement-mediated lysis as the indicator system.

   That Coombs serum identifies only anti-Rh antibodies (**choice C**) is not true. Coombs serum is antihuman IgG. It will bind to the Fc portion of any human IgG molecule, regardless of its antigenic specificity.

   The statement that the high titer of natural isohemagglutinins makes Coombs reagent unnecessary (**choice E**) is not true. It is the isotype of these antibodies (IgM) and the size of that molecule that allows agglutination to proceed without a developing antibody.

5. **The correct answer is A.** The direct fluorescent antibody test is used to detect antigens in the tissues of a patient.

   The enzyme-linked immunosorbent assay (**choice B**) is a test usually used to detect antibody production. It can be modified to detect antigen, but not from a tissue specimen such as this one.

   The indirect fluorescent antibody test (**choice C**) is used to detect antibodies being produced in a patient. It is not used for detection of microbial antigens.

   Radioimmunoassay (**choice D**) is used to detect antibodies in a patient, not antigen.

   Western blot (**choice E**) is used to detect antibodies in a patient, not antigen.

CD Markers

CD Designation	Molecular Family	Cellular Expression	Known Functions
CD2 (LFA-2)	Ig superfamily	T cells, thymocytes, NK cells	Adhesion molecule
CD3	Ig superfamily	T cells, thymocytes	Signal transduction by the TCR
CD4	Ig superfamily	TH cells, thymocytes, monocytes, and macrophages	Signaling and adhesion coreceptor in Ag-induced TH-cell activation, receptor for HIV
CD8	Ig superfamily	CTLs, some thymocytes	Coreceptor for MHC class I–restricted T cells
CD14 (LPS receptor)		Monocytes, macrophages, granulocytes	Binds LPS
CD16 (Fc receptor)	Ig superfamily	NK cells, macrophages, mast cells	Immune complex–induced cellular activation, ADCC
CD18	β chain of LFA-1 family, β_2 integrin subunit	Leukocytes	Complement receptor and cell adhesion molecule
CD19	Ig superfamily	B cells	Coreceptor with CD21 for B-cell activation
CD20	Tetraspan family	Most or all B cells	Unknown role in B-cell activation
CD21 (CR2, C3d receptor)		Mature B cells, follicular dendritic cells	Receptor for complement fragment C3d, forms coreceptor complex with CD19, Epstein-Barr virus receptor

(*Continued*)

CD Designation	Molecular Family	Cellular Expression	Known Functions
CD28	Ig superfamily	T cells	T-cell receptor for costimulatory molecule B7
CD34	Sialomucin	Precursors of hematopoietic cells, endothelial cells in HEV	Cell–cell adhesion, binds L-selectin
CD40	TNF-R family	B cells, macrophages, dendritic cells, endothelial cells	Binds CD40L, role in T-cell–dependent B cell, macrophage, dendritic cell and endothelial cell activation
CD45 (Leukocyte common antigen)	Tyrosine phosphatase	Hematopoietic cells	Plays critical role in T- and B-cell antigen receptor-mediated signaling
CD56		NK cells	Not known

APPENDIX

Cytokines

Cytokine	Secreted by	Target Cell/Tissue	Activity
Interleukin (IL)-1	Monocytes, macrophages, B cells, dendritic cells, endothelial cells, others	TH cells	Costimulates activation
		B cells	Promotes maturation and clonal expansion
		NK cells	Enhances activity
		Endothelial cells	Increases expression of ICAMs
		Macrophages and neutrophils	Chemotactically attracts
		Hepatocytes	Induces synthesis of acute-phase proteins
		Hypothalamus	Induces fever
IL-2	TH cells	Antigen-primed TH and CTLs	Induces proliferation, enhances activity
IL-3	TH cells, NK cells	Hematopoietic cells	Supports growth and differentiation
IL-4	TH2 cells	Antigen-primed B cells	Costimulates activation
		Activated B cells	Stimulates proliferation and differentiation, induces class switch to IgG1 and IgE
		Resting B cells	Upregulates class II MHC expression
IL-5	TH2 cells	Activated B cells	Stimulates proliferation and differentiation, induces class switch to IgA

(*Continued*)

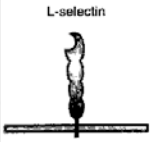
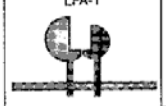
Cytokine	Secreted by	Target Cell/Tissue	Activity
IL-6	Monocytes, macrophages, TH2 cells, bone marrow stromal cells	Proliferating B cells	Promotes terminal differentiation into plasma cells
		Plasma cells	Stimulates Ab secretion
		Myeloid stem cells	Helps promote differentiation
		Hepatocytes	Induces synthesis of acute-phase proteins
IL-7	Bone marrow, thymic stromal cells	Lymphoid stem cells	Induces differentiation into progenitor B and T cells
IL-8	Macrophages, endothelial cells	Neutrophils	Chemokine, induces adherence to endothelium and extravasation into tissues
IL-10	TH2 cells	Macrophages	Suppresses cytokine production by TH1 cells
IL-12	Macrophages, B cells	Activated CD8+ cells	Acts synergistically with IL-2 to induce differentiation into CTLs
		NK and LAK cells and activated TH1 cells	Stimulates proliferation
Interferon-α	Leukocytes	Uninfected cells	Inhibits viral replication
Interferon-β	Fibroblasts	Uninfected cells	Inhibits viral replication
Interferon-γ	TH1, CTLs, NK cells	Macrophages	Enhances activity
		Many cell types	Increases expression of classes I and II MHC
		Proliferating B cells	Induces class switch to IgG2a, blocks IL-4–induced class switch to IgE and IgG1
		TH2 cells	Inhibits proliferation
		Inflammatory cells	Mediates effects important in DTH

(*Continued*)

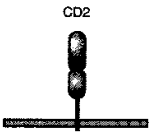
Cytokine	Secreted by	Target Cell/Tissue	Activity
Transforming growth factor-β	Platelets, macrophages, lymphocytes, mast cells	Proliferating B cells	Induces class switch to IgA
Tumor necrosis factor-α	Macrophages, NK cells	Tumor cells	Has cytotoxic effect
		Inflammatory cells	Induces cytokine secretion, causes cachexia of chronic inflammation
Tumor necrosis factor-β	TH1 and CTL	Tumor cells	Has cytotoxic and other effects, like TNF-α
		Macrophages and neutrophils	Enhances phagocytic activity
Granulocyte colony-stimulating factor (G-CSF)	Macrophages and TH cells	Bone marrow granulocyte precursors	Induce proliferation, used clinically to counteract neutropenia following ablative chemotherapy
Granulocyte–macrophage colony-stimulating factor (GM-CSF)	Macrophages and TH cells	Bone marrow granulocyte and macrophage precursors	Induces proliferation; used clinically to counteract neutropenia following ablative chemotherapy

APPENDIX

Adhesion Molecules

Family	Figures	Name	Tissue Distribution	Ligand
Selectins (bind carbohydrates, initiate leukocyte–endothelial interaction)		L-selectin	Leukocytes	Addressins
		P-selectin	Endothelium and platelets	Addressins
		E-selectin	Activated endothelium	Addressins
Mucin-like vascular **addressins** (bind to L-selectin, initiate leukocyte endothelial interaction)		CD34	Endothelial venules	L-selectin
		GlyCAM-1	High endothelial venules	L-selectin
		MAdCAM-1	Mucosal lymphoid tissue venules	L-selectin
Integrins (bind to cell-adhesion molecules and extracellular matrix; strong adhesion)		LFA-1	Monocytes, T cells, macrophages, neutrophils, dendritic cells	ICAMs
		CR3	Neutrophils, monocytes, macrophages	ICAM-1, iC3b, fibrinogen
		CR4	Dendritic cells, macrophages, neutrophils	iC3b

(*Continued*)

Family	Figures	Name	Tissue Distribution	Ligand
Immunoglobulin superfamily (various roles in cell adhesion, ligand for integrins)		CD2	T cells	LFA-3
		ICAM-1	Activated vessels, lymphocytes, dendritic cells	LFA-1, CR3
		ICAM-2	Resting vessels, dendritic cells	LFA-1
		ICAM-3	Lymphocytes	LFA-1
		LFA-3	Lymphocytes, antigen-presenting cells	CD2
		VCAM-1	Activated endothelium	VLA-4

APPENDIX IV

Mechanisms of Resistance to Microbial Infections

Category	Pathogen	Protective Mechanism	Evasive Mechanism	Immune Pathology
Prion	CJD, kuru, etc.	No immune response	None	None
Viruses	Naked capsid (all)	Antibody blocks receptor binding		
	Rhinovirus	See above	Antigenic drift	
	Adenovirus	See above	Decrease MHC I expression	
	Enveloped (all)	Antibody blocks receptor binding, C lyses envelope, Ab and C enhances phagocytosis, TH1 cells stimulate CTLs and NK		
	Hepatitis C		Blocks IFN-α and -β	
	Hepatitis B			Immune complexes cause vasculitis
	All Herpesviridae		Nuclear membrane envelope is nonimmunogenic	
	Herpes simplex 1 and 2		Block TAP function (inhibits MHC I expression), viral glycoprotein ↓ C activation	

(*Continued*)

Category	Pathogen	Protective Mechanism	Evasive Mechanism	Immune Pathology
	Cytomegalovirus		Generalized immuno-suppression, ↓ MHC I and II expression, produces chemokine receptor and MHC I homologues	
	Epstein-Barr virus		Generalized immuno-suppression, produces molecule homologous to IL-10 (shuts down TH1)	
	Paramyxoviruses		Generalized immuno-suppression	
	Human Immuno-deficiency virus		Infects and kills immuno-competent cells, antigenic drift	
	Rubeola		↓ MHC II expression	
	Influenza		Antigenic shift and drift	
Extracellular bacteria	All	Ab or C enhance phagocytosis or block toxin binding		
Gram-negative extracellular bacteria		C opsonizes		LPS activates macrophages → IL-1 and TNF-α
	Neisseria gonorrhoeae		Antigenic variation of pili	
	Pseudomonas		Inactivates C3a, C5a	

(*Continued*)

Category	Pathogen	Protective Mechanism	Evasive Mechanism	Immune Pathology
	Borrelia burgdorferi			Immune complexes cause rash, arthritis, neurologic symptoms
Encapsulated bacteria	All	Opsonization with Ab and C	Resist phagocytosis	
IgA protease producers	*Streptococcus pneumoniae, Haemophilus influenzae, Neisseria meningitidis* and *Neisseria gonorrhoeae*		Destroy IgA	
Coagulase-positive bacteria	*Staphylococcus aureus* *Yersinia pestis*		Inhibit phagocytosis	
Gram-positive, extracellular bacteria	*Streptococcus pyogenes*			Exotoxins act as superantigens, nonsuppurative sequelae: types II and III hypersensitivities
Catalase-positive bacteria	All		Catalase destroys reactive oxygen intermediates	
	Staphylococcus aureus			Exotoxins act as superantigens
Intracellular bacteria		CD4+ cells activate NK, macrophages, and CTLs		
	Mycobacterium tuberculosis	See above	Sulfatides inhibit phagolysosome formation	Granuloma formation is CMI-mediated
	Mycobacterium leprae	TH1 stimulation is protective (tuberculoid form)	TH2 stimulation results in lepromatous form	Nerve damage in tuberculoid form is DTH-mediated

(*Continued*)

Category	Pathogen	Protective Mechanism	Evasive Mechanism	Immune Pathology
	Listeria monocytogenes	DTH and CTL	Hemolysin disrupts phagosome membrane, allows escape into cytoplasm	
	Chlamydia trachomatis	DTH and CTLs		Scarring of fallopian tubes is DTH-mediated
Extracellular protozoa	All	Abs to surface molecules plus C cause lysis or opsonize		
	Trypanosoma brucei rhodesiense and *gambiense*	See above	Antigenic variation of variable surface glycoprotein	
	Entamoeba histolytica	See above	Antigen shedding	
Intracellular protozoa		TH1 cells stimulate phagocytosis, CTLs		
	Plasmodium spp.	Antibodies plus above	Maturational stages change antigens, antigenic variation	
	Trypanosoma cruzi	Amastigotes killed by CMI, trypomastigotes (extracellular) killed by Ab and C		
	Leishmania spp.	TH1 cells stimulate phagocytosis, CTLs		

(*Continued*)

Category	Pathogen	Protective Mechanism	Evasive Mechanism	Immune Pathology
Helminth parasites	All	ADCC-mediated by eosinophils, macrophages		
	Filarial nematodes		Immuno-suppression secondary to lymphatic obstruction	
	Schistosoma spp.		Adult envelopes itself in host self glycoproteins (ABO and MHC antigens)	Granulomas in liver due to DTH response to egg antigens
Fungi	Most	Phagocytosis by PMNs and macrophages, Abs not protective		
	Cryptococcus neoformans	As above	Inhibits TH1, stimulates TH2, capsule protects against phagocytosis	
	Histoplasma capsulatum	TH1 cell stimulates CTLs and NK		

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